Pulp and Paper Microscopy

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THIRD EDITION
SECOND PRINTING

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PREFACE

Fifteen years have passed since the second edition of John H. Graff's "Pulp and Paper Microscopy" (commonly known as "Fiber Analysis") was published. Like its forerunner it was received favorably by the industry. A new edition is sorely needed. Mr. Graff, after his retirement as group leader in microscopy at The Institute of Paper Chemistry in late 1946, expended considerable energy to this end but was prevented from completing the task by his death in December, 1949. The pulp, paper and paperboard industry is deeply indebted to him for his untiring efforts to increase the use of the microscope in mill and laboratory.

The present author has referred to the notes and manuscript of the previous author frequently. In addition, my colleagues and other experts have been consulted for advice and criticism. I desire to express my sincere appreciation to all who have assisted in the revision of this monograph.

Irving H. Isenberg

Appleton, Wisconsin

November, 1957

PREFACE TO SECOND PRINTING

With the need for a new printing of the Third Edition of Pulp and Paper Microscopy an attempt has been made to bring all chapters up-to-date by including additional information and references. These minor additions should enhance the value of the book as a reference and text.

Irving H. Isenberg

Appleton, Wisconsin

June, 1967

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CHAPTER I

ELEMENTARY PRINCIPLES OF MICROSCOPE OPTICS

The microscopist, like any efficient technician, must be well-versed in the design and operation of his tools. It is beyond the scope of this book to give a lengthy discourse on geometrical and physical optics or a thorough explanation of the use and construction of the microscope. Numerous authors have done so (1-16). It is deemed necessary, however, to present briefly in review certain of the basic principles in order to orient the average laboratory worker in the industry.

LIGHT AND THE PRINCIPLES OF LENSES

REFLECTION OF LIGHT

The angle of reflection always is equal to the angle of incidence, and the plane including the incident and reflected rays is perpendicular to the reflecting surface.

If the incident light strikes an uneven surface instead of a polished one, some of the light will be reflected and the remainder will be scattered (diffused reflection).

REFRACTION OF LIGHT

The magnification power of all microscopes in ordinary use, whether simple or compound, depends upon the ability possessed by lenses to refract or bend the light which passes through them. Refraction acts in accordance with the following natural laws:

1. A ray, which in passing from a rarer medium into a denser medium makes a certain angle with the normal (i.e., the perpendicular to the surface or plane at which two media join), will, on entering the denser medium, make a smaller angle with the normal. Conversely, a ray passing from a denser medium into a rarer one, making a certain angle with the normal, will on emerging from the denser medium, make a larger angle with the normal.

The ray in one medium is called the incident ray and in the other medium the refracted ray. The incident and refracted rays are always in the same plane as the normal to the refracting surface at the point of incidence.

2. The sine of the angle of incidence divided by the sine of the angle of refraction is a constant quantity for any two media.

The ratio sin i/ sin r is called n, the index of refraction. The angle of incidence is usually measured in air. When one of the media is air (more correctly, a vacuum), the ratio of these sines is called the absolute index of refraction. The relative index of refraction for a ray of light passing from one medium to another is found by dividing the absolute index of the medium into which it is passing by that from which it is coming. It was shown by Huygens that refraction is very simply explained by assuming a change of velocity in passing from one medium to another.

DISPERSION OF LIGHT

A ray of white light passing through a prism is dispersed or separated into a continuous spectrum. This occurs because the shortest waves, those which produce the sensation of violet color, are retarded most and hence refracted most whereas the longest waves, those which produce the sensation of red color, are retarded least and consequently refracted least. It follows that the index of refraction for any substance will vary according to the color employed. The angular separation or dispersion between two colors depends on the difference between their respective indices of refraction. The difference between the values for red and violet light determines what is called the dispersive power of the substance.

There are great differences between the refractive and dispersive powers of different specimens of glass. For example, the refractive index for a red ray in heavy flint glass is 1.7030 and for a violet ray is 1.7501 which results in a dispersive power of 0.0471. Crown glass has corresponding values of 1.5124, 1.5288, and 0.0164. These differences are used in the construction of achromatic and apochromatic lenses.

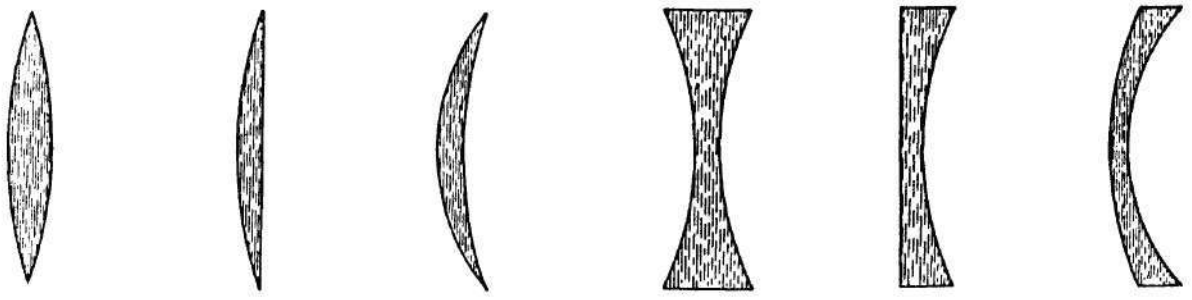
LENSES

A lens is defined as a transparent body, usually a piece of glass, having two opposite regular surfaces, either both curved, or one curved and the other plane, with a common normal.

Lenses are divided into two classes. The first class, convex or converging lenses, cause a beam of parallel rays to converge; the second class, concave or diverging lenses, cause a beam of parallel rays to diverge. Figure 1 illustrates the principal sections of some typical lenses.

Parallel rays of white light striking a convex lens form real images (positive or convergent); the same light striking a concave lens forms virtual images (negative or divergent).

The principal effects of lenses on light incident on the surfaces are as follows:



1a. Biconvex, Plano-convex, and Converging Meniscus Lenses

1b. Biconcave, Plano-concave, and Diverging Meniscus Lenses

Figure 1

1. If a radiant is placed at the principal focus of a converging lens the rays are rendered parallel; conversely, if parallel rays fall on a converging lens they are brought to a principal focus or point upon the axis (Figure 2).

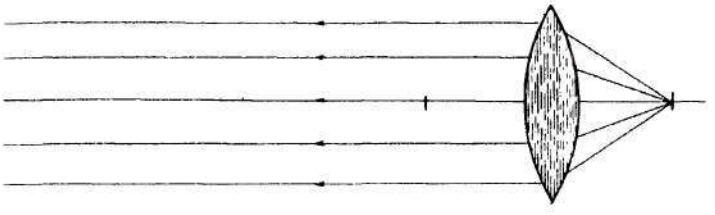


Figure 2. A Radiant at the Principal Focus of a Biconvex Lens Makes the Refracted Rays Parallel

2. If a radiant is placed beyond the principal focus of a converging lens the rays are brought to a focus beyond the principal focus on the other side of the lens. The nearer the radiant is to the principal focus, the further away will be the conjugate focus from the other principal focus (Figure 3).

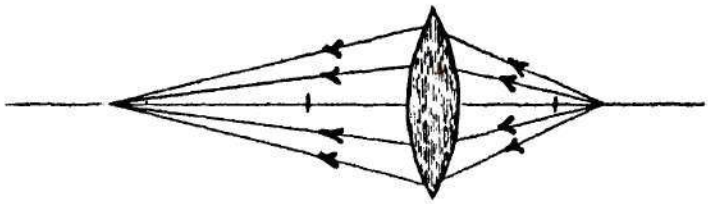


Figure 3. A Radiant Placed Beyond the Principal Focus Causes Rays to Converge Beyond the Principal Focus on the Other Side of the Lens

Should the radiant be at a distance from the principal focus equal to the focal length of a positive lens (i.e., twice the focal length from the lens) its conjugate will be at the same distance from the focus on the other side of the lens. In other words, when the object and its image are equidistant on either side of a positive lens they are equal in size and are four times the focal length plus the distance between nodal points of the lens apart. (In general, only for a thin lens.)

3. If a radiant is placed between a lens and its principal focus, the rays on the other side of the lens are still divergent but if these rays are traced backwards they will then be directed toward a point called the virtual conjugate focus (Figure 4).

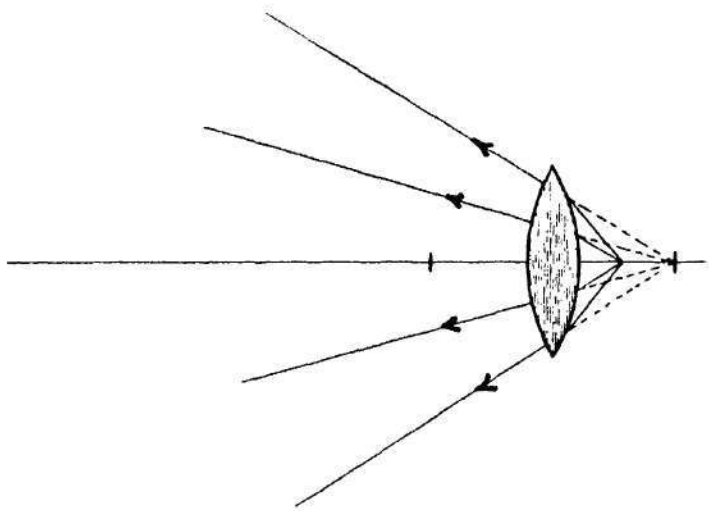


Figure 4. Rays Diverge When a Radiant is Placed Between a Lens and its Principal Focus. Focus of Divergent Rays is Virtual

4. The image from a concave lens is not real but virtual. (A real image can be received on a screen but a virtual image cannot.) Parallel rays falling on a concave lens are rendered divergent on the other side of the lens and consequently can never come to a focus. However, when these divergent rays are traced backwards they are directed toward a point known as the virtual principal focus of the lens (Figure 5).

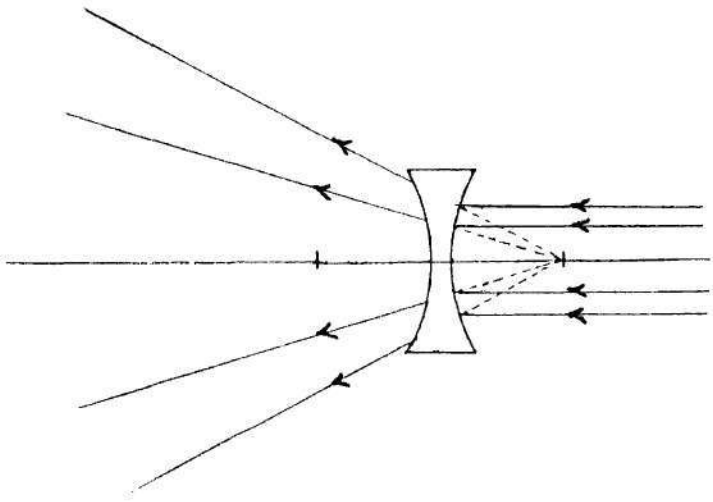


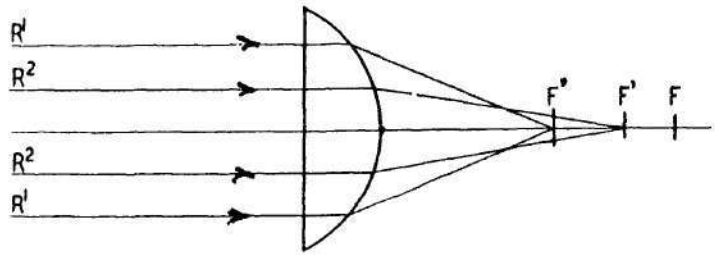
Figure 5. Virtual Focus of a Concave Lens

SPHERICAL ABERRATION

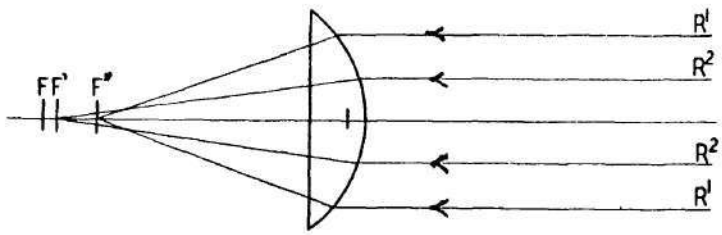
The light which goes through the edges of a converging lens is converged a little more than that which goes through the center so that it does not come to the same focus. The result is that images formed by a wide lens are somewhat blurred, an effect called spherical aberration. The covering of the outer part of the lens by a diaphragm will greatly reduce spherical aberration. It is also possible to grind surfaces slightly differing from a spherical form, so that for a given pair of conjugate focal distances the emergent wave is truly spherical. These are aplanatic lenses (Figure 6).

CHROMATIC ABERRATION

If parallel white light strikes a convex lens the unequal refraction of various wavelengths is such that the most refracted of its component rays



6a. Spherical Aberration



6b. Spherical Aberration

Figure 6

(the violet) will be brought to a focus at a point somewhat nearer the lens than the principal focus and the least refracted (the red) will be brought to a focus at a point farther from the lens than the principal focus which is, in effect, the mean of the chromatic foci (Figure 7).

IMAGE FORMATION

Two kinds of images are formed by lenses--a real image and a virtual image. The formation of a real image means the production of a picture by a lens, or a combination of lenses, which can be cast upon a screen. Examples of real images are those produced by a projection lantern and by a camera upon the focusing glass (Figure 8). When it cannot be received on a screen, for example, the image seen through a hand lens or what may be called "the simple microscope", an image is said to be virtual (Figure 9).

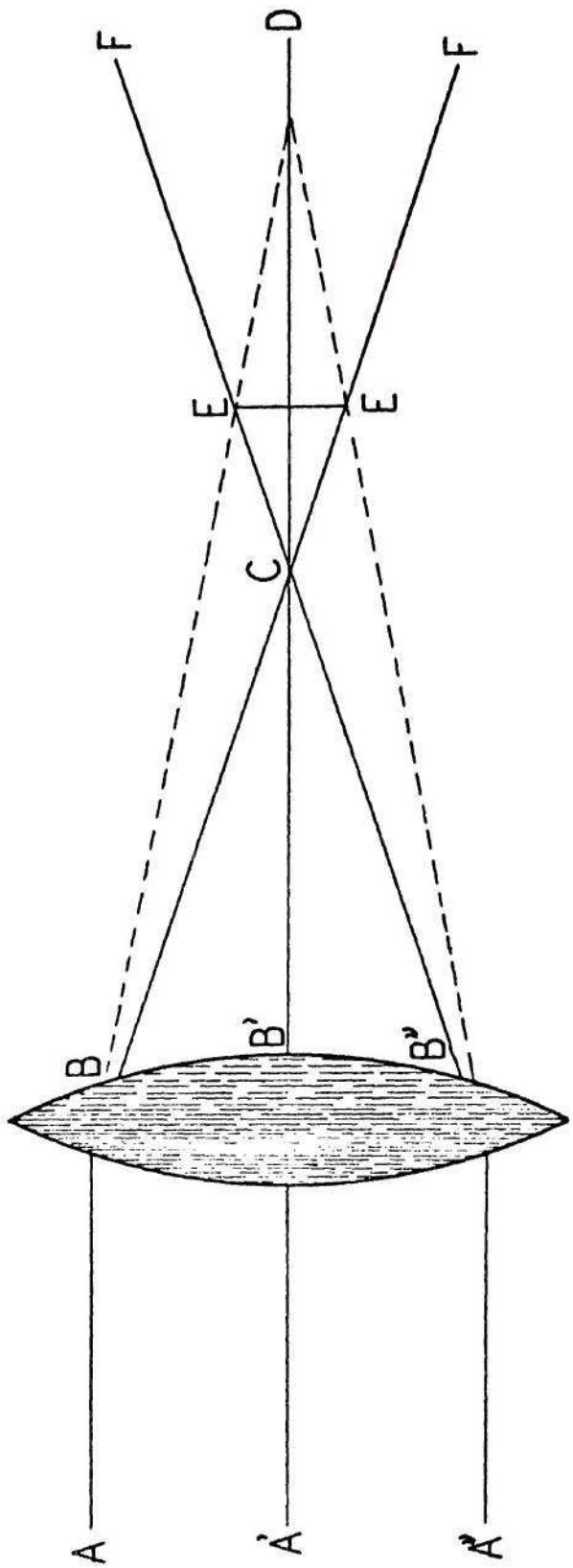


Figure 7. Chromatic Abberation

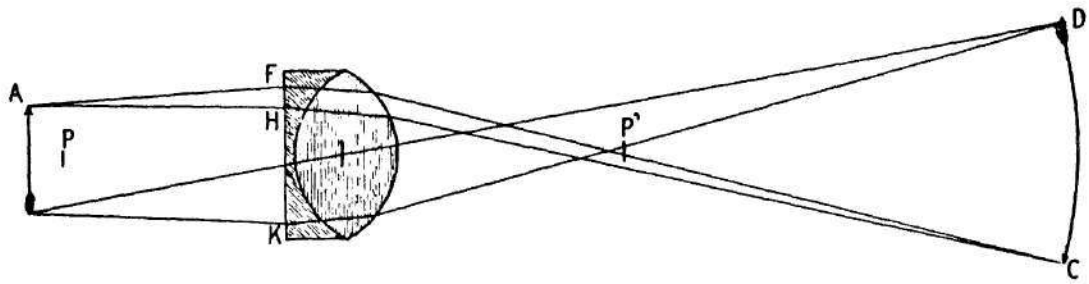


Figure 8. The Formation of a "Real Image"

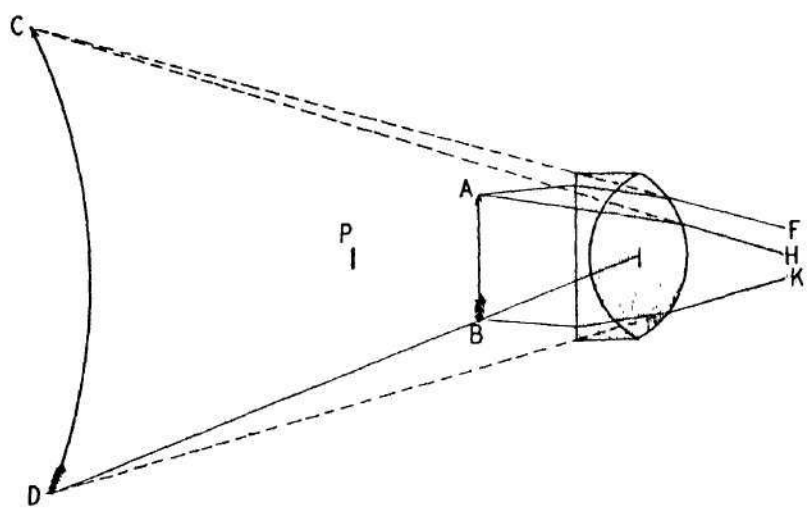


Figure 9. The Formation of a "Virtual Image"

THE OPTICAL SYSTEM OF THE COMPOUND MICROSCOPE

IMAGE FORMATION

The simplest form of a compound microscope consists of two convex lenses: one small and placed near the object, the other larger and placed in such a position that the image formed by the small lens is in its focus. The smaller lens is called the objective because it is placed near the object; the larger lens is known as the eyepiece or ocular because the eye is placed near it to make observations (Figure 10).

In practice, an objective consists of several lenses placed close together. The lens nearest the object is called the front lens or front lens combination; the one farthest from the object is called the back combination. These lenses act together to magnify the object. Two or more lenses of slight curvature are used rather than one considerably curved as it is found that spherical aberration and dispersion are thus reduced to a minimum.

The eyepiece sometimes consists of a single convex lens. It is then known as a positive eyepiece and is really a simple microscope. More commonly a Huygenian eyepiece is used. This consists of two convex lenses. The one nearer the eye is called the eye lens; the other, the field lens. The field lens acts precisely as one of the lenses of an objective and is regarded by many as constituting a part of the objective (Figure 11).

The diaphragm of the eyepiece is in the plane in which the image is formed. When an eyepiece micrometer is used it is placed in this position. So also is a pointer or crosshairs. The eye lens acts as a simple microscope and magnifies this image.

MAGNIFICATION

The size of the real, inverted image is determined by the size of the object and the relative distance of the object and the image from the objective. This relation, in its simplest form, is expressed by the following equation for the magnification by the objective:

$$\frac{\text{optical tube length}}{\underline{f}\ \text{objective}},$$

where $\underline{f}$ = the focal length of the objective.

Increasing or decreasing the mechanical tube length between the usual limits of 140 to 180 mm. can vary the magnification by 25%.

Figure 10. Formation of Image by Simplest Form of Compound Microscope

Figure 11. Formation of Image by Compound Microscope with Two-lens Eyepiece

The eyepiece magnification can be expressed as follows:

$$\frac{250 \text{ mm.}}{f \text{ eyepiece}}$$

where 250 mm. is the arbitrarily assumed closest viewing distance of a normal unaided eye, and f = the focal length of the eyepiece.

$$\text{Total magnification} = \frac{\text{optical tube length}}{f \text{ objective}} \times \frac{250 \text{ mm.}}{f \text{ eyepiece}}$$

PARTS OF THE MICROSCOPE

In its simplest form the microscope can be considered to consist of four essential parts: the stand which holds a tube in which is situated the system of lenses--objectives and eyepieces, a stage on which the object is placed, and a substage which is provided with auxiliary parts for the illumination of the object (Figure 12).

Objectives

The purpose of the objective is to gather the light coming from a point of the object to be examined and to assemble it in a point of the image at a distance so that magnification is achieved.

Resolution. A property of greater importance than the magnification is the resolution or resolving power of the objective. The ability of an objective to delineate details in an object is dependent not only upon the magnification power but upon the angle of the cone of rays which enter the objective from a point in the object.

The angular magnitude of the cone of rays entering the objective indicates the power of the objective to reveal fine structures in the object. It has been found, however, that the measurement of the resolving power by the number of degrees in the angle of the aperture is not exact.

Abbé showed that the index of refraction (n) of the immersion medium must be taken into account in determining the resolving power of an objective. He proposed the term numerical aperture (N.A.).

$$\text{N.A.} = n \sin \frac{AA'}{2}$$

where AA' = the angular aperture of a lens diameter as viewed from an object point. The numerical aperture is a true measure of the resolving power of an objective.

Diffraction theory of resolution. The resolving power of a microscope objective may be defined as its ability to reveal closely adjacent structural

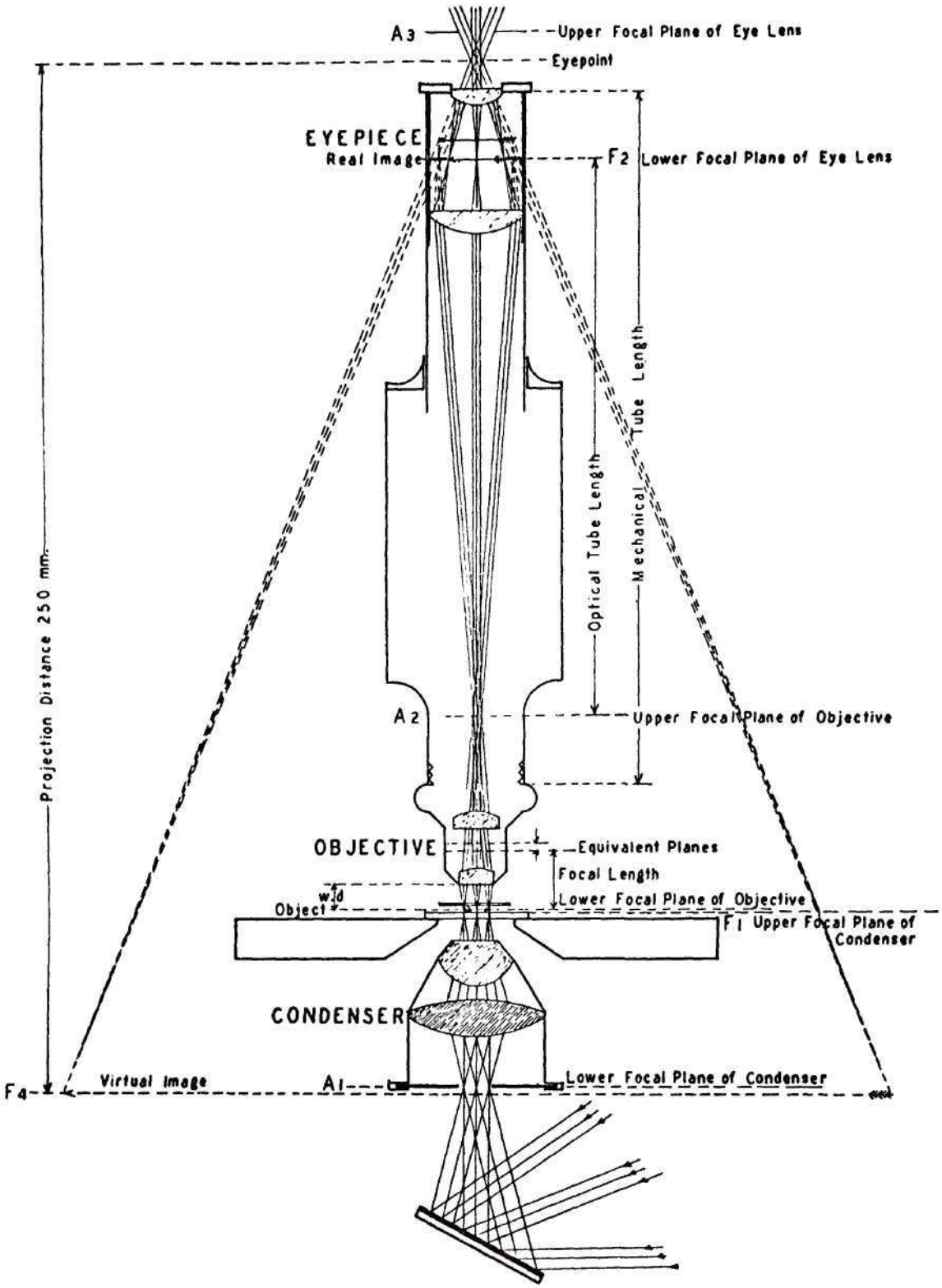


Figure 12. The Optical System of the Microscope

details as actually separated and distinct. Quantitatively, it may be expressed as the minimum distance between such details when resolution is achieved.

If a diffraction grating is illuminated by a beam of light perpendicular to its surface, spectra are formed on either side of the path of the directly transmitted ray. The angle which the diffracted ray makes with the central beam depends on the distance between the lines of the grating in comparison with the wavelength of light in a medium of refractive index n.

A grating surrounded by a highly refractive medium diffracts light through a smaller angle than it does in a medium of low refractive index.

Increase of resolution of an objective. 1. Resolution can be increased by using light of short wavelength since

$$\text{Resolving power} = \frac{1.3\ \lambda}{2\ \text{N.A.}},$$

where λ is the wavelength of light and 1.3 is a factor which is necessary because an objective is less efficient in practice than its stated angular aperture indicates.

2. Resolution can be increased by the use of immersion objectives in immersion fluids with a high refractive index (Figure 13).

3. Lastly, resolution can be increased by using oblique illumination because, according to Abbé's theory, the direct ray or one of the diffracted rays should be sufficient to resolve a grating. By the use of an oblique beam, both of these two rays may be grasped by the objective. It is possible, with the proper degree of oblique illumination, to nearly double the resolving power of the objective.

Importance of numerical aperture. With a very narrow pencil of light the resolution is equal to $\frac{1.3\ \lambda}{\text{N.A.}}$ but if the light is made to fill the whole aperture of the objective the distance between adjacent lines is halved and the resolution is equal to $\frac{1.3\ \lambda}{2\ \text{N.A.}}$.

$$\text{Optimum N.A.} = \frac{\text{N.A. of objective} + \text{N.A. of condenser}}{2}$$

Depth of focus. When an object is in sharp focus, very little of the object immediately above or below the point actually in focus can be seen at the same time. The ability of the objective to show these different planes is called the penetrating power or depth of focus of the objective. This penetrating power decreases very rapidly with increase in the magnification power of the objective.

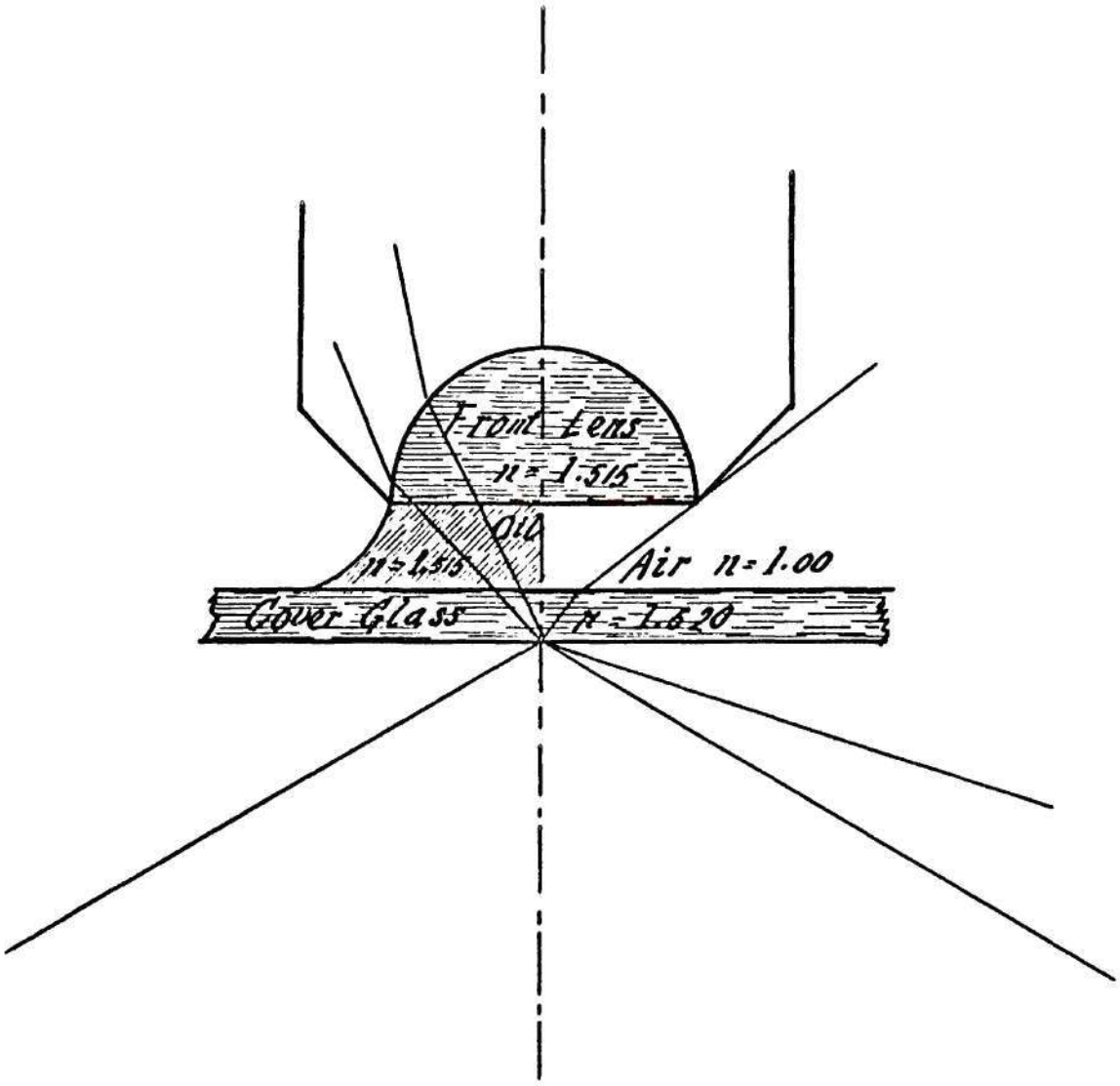


Figure 13. Increased Numerical Aperture with Immersion Objectives

One means of determining the penetrating power of an objective is by the following equation:

$$D = \frac{ns}{(N.A.)\ M}$$

where D is the depth of focus, n the index of refraction, s the diameter of the circle of confusion, and M the magnification; s is generally accepted as 0.254 mm.

Spherical and chromatic aberration of objectives. Since the rays in passing through lenses of various kinds are unequally refracted, they cannot all meet exactly in a single focal plane. This can be overcome by reducing the aperture of the objective at the expense of the resolving power.

Chromatically corrected objectives may give slightly different magnifications with light of different wavelengths. These chromatic differences of magnification will show as color bands near the edge of the field and special eyepieces, possessing a similar but opposite chromatic difference of magnification, are made to neutralize this aberration. Such eyepieces are called compensating eyepieces and should always be used with these objectives.

Types of microscope objectives. Achromatic objectives are corrected chromatically for two colors (green and red) and corrected spherically for one color (green).

Apochromatic objectives are corrected chromatically for three colors (violet, green, and red) and spherically corrected for two colors (green and violet).

Fluorite or semiapochromatic objectives are objectives of a higher order of correction than the achromatics. The secondary spectrum is reduced to such an extent that the correction of these objectives lies about halfway between that of the achromatic and apochromatic types.

U.V. objectives are corrected for two wavelengths (365 nm. and 546 nm). The focusing of the object is done at the wavelength of 546 nm. and the photomicrography in ultraviolet light at about 365 nm.

Micro Tessar objectives are short focal length lenses for preparing photomicrographs of a comparatively large area of a specimen at low magnification. These lenses are corrected for use without an eyepiece.

Immersion objectives. The increased numerical aperture possible with immersion objectives makes them advantageous when the highest possible resolution is needed. The medium of the lowest refractive index between the object and the condenser is the limiting factor and the effective N.A. of the objective will always be less than this value.

Methods of testing objectives. When an objective is made it is corrected for a special tube length which in some cases is engraved on the mount. The

tube length is corrected only for the special object and cover glass thickness which the manufacturer uses and, speaking generally, it will be found that the particular tube length will not be precisely the same for another object which may be examined.

The question of correct tube length is the most important in microscopy and it is essential to have such knowledge before any lens can be tested or before the best can be obtained from an objective. Another important point is that an objective cannot be effectively tested unless it is illuminated with a condenser of nearly the same numerical aperture as the object glass.

Abbé Test Plate (The Star Test). This consists of a silvered glass slide ruled with a number of fine parallel lines and covered with a cover glass, the thickness of which increases from one end of the slide to the other (0.09 to 0.24 mm.).

This is mounted on the stage and examined when illuminated by a condenser whose numerical aperture is equal to that of the objective. The following observations can be made:

1. Test for Tube Length and Spherical Aberration. If, in focusing downwards, rings appear but disappear when focusing above the correct focus, it follows that the objective is undercorrected for spherical aberration and the tube length should be increased. The rule is: when rings appear on focusing downwards, lengthen the tube; when rings appear on focusing upwards, shorten the tube.

If the rings are elliptical, the lenses are not corrected for astigmatism. If the rings are circular but not concentric, the lens elements are not properly centered.

2. Zonal Aberration. Excessive inequality of the spacing of the rings above and below the focus indicates a need of zonal correction.

3. Chromatic Aberration. Lack of chromatic correction of the objectives will show marginal blue and red fringes.

4. Cover Glass Correction. If the cover glass is too thin, increase the tube length; if it is too thick, decrease the tube length.

Eyepieces

The chief functions of the eyepiece (ocular) are to form a magnified real image of the real image from the objective on the retina of the eye or on a photographic plate, and to image scales, crosshairs, pointers, or other objects located within the eyepiece.

Eyepieces, as a rule, are designated by their magnification numbers (250 mm./f) rather than by their focal length.

A diaphragm is placed at the lower focal plane of the eyepiece which delimits the field of view. Micrometer scales, crosshairs and pointers when used are placed at this level.

Since the angular aperture of the lenses of the eyepieces is relatively small, the aberrations are not as serious as are those of the objectives.

Eyepieces may be classified into four main groups: negative (Huygenian and Hyperplane) which has the image plane between the eye lens and the field lens making the field lens essentially a part of the objective system; positive (Ramsden) which has the image plane below the field lens permitting the use of this eyepiece as a magnifying glass; compensating eyepieces which are used in conjunction with apochromatic objectives to eliminate color fringes; and amplifying eyepieces (Ampliplane or Homal) which are used to give a flat field in photomicrography. In addition, there are various special types such as demonstration eyepieces, comparison eyepieces, erecting eyepieces, and wide-angle eyepieces.

To secure the best optical performance of standard microscope eyepieces and objectives, the most satisfactory type of eyepiece to be used with each objective is listed in Table I. It will be noted that Huygenian eyepieces are used only with achromatic objectives.

Limit of magnification and resolving power of the microscope. When well-corrected lenses are used, the magnifying power of the microscope should be at least that necessary to reveal the finest details resolvable by the object. For the normal eye, this is equivalent to 500 to 700 times the numerical aperture of the objective. The limit of useful magnification for visual work should not be greater than 1000 x N.A. In photomicrography, the limit may be higher because of the short wavelengths of light used. By the use of ultra-violet light, maximum resolution has been obtained and magnifications as high as 3000 to 4000 have been permissible. Practically speaking 0.2 μm. may be considered the limit of resolving power for visual light and about 0.1 μm. for ultraviolet light.

The limit of visibility. The limit of visibility is a measure of the smallest particles which may be seen. It is less than the limit of resolution and varies widely depending on the proximity of the particles, their color and refractive index and, in particular, whether bright or dark field illumination is used.

Condensers

The chief functions of the condenser are to concentrate light upon the object and increase the brilliance of the image, to furnish oblique and dark field illumination, and to project images or scales in the plane of the object. In critical microscopy at high powers the condenser has the additional and highly essential function of supplying strongly convergent light to the object as an aid in resolution. With vertical illumination the objective serves as a condenser and has similar functions.

TABLE I

EYEPIECES FOR DIFFERENT OBJECTIVES

Objective	E.F. in mm.	Type of Eyepiece
Achromatic	48.0	Huygenian
	40.0	Huygenian
	32.0	Huygenian
	16.0	Huygenian
	8.0	Hyperplane
	7.0	Hyperplane
	4.0	Hyperplane
	3.0	Hyperplane
	1.9 (oil)	Compensating
Fluorite	4.0	Hyperplane
	1.8 (oil)	Hyperplane
	1.8 (oil)	Compensating
Apochromatic	16.0	Compensating
	8.0	Compensating
	4.0	Compensating
	3.0	Compensating
	2.0 (oil)	Compensating
	1.5 (oil)	Compensating

Concentration of light by condensers. A condenser acts, as does any lens, to form an image at or just outside its focal point. The size of the image of the light source is governed by the ratio of its distance to the distance at which its image is formed. The condenser thus forms a reduced image roughly focal length/d as large as the light source. The reduced image is correspondingly more brilliant than the original light source.

The angular aperture of most condensers is fairly large so that the light is strongly convergent at the point of focus. This convergency may be decreased by the use of an iris diaphragm. Hence, the intensity and also the obliquity of the illumination may be adjusted.

The focal length. The focal length of the condenser is important. If the focal length is too short, the image of the light source will be too small to cover the field of the objective. Most condensers are constructed with the top part removable so the remainder of the lens combination, having a longer focal length is able to illuminate a larger area.

The working distance. In high aperture condensers the working distance is much less than its focal length. Selected thin slides are required if the working distance is less than approximately 1.5 mm.

Oblique illumination by condensers. Unilateral oblique illumination is obtained when only one side of the condenser aperture is left open (the rest being closed by an opaque screen). The degree of obliquity is determined by the angular aperture of the condenser and also to the extent by which the axial rays are stopped out.

Dark field illumination. The use of an opaque disk (central stop) placed in the center of the diaphragm of the condenser forms an annular aperture through which a hollow cone ray passes to the objective. The size of the stop must be such as to cut off all direct rays from entering the objective. Under this condition "annular or dark field illumination" is obtained and the surface of the object appears selfluminous against a dark background.

Projection of images by condensers. Just as the image of the light source is projected in the plane of the preparation, in a similar manner other objects may be imaged in the plane of the preparation. Diaphragms to regulate the area illuminated, special scales for micrometry, or color standards for comparison may be superimposed upon the image of the object.

Types of substage condensers. The Abbé Condenser. The most commonly used is the divisible type with 1.20 N.A., which consists of two elements; the upper element is removable. A dark-field element can be substituted for the upper element.

The Abbé Condenser 1.40 N.A., a three-element system, is not divisible.

The Aplanatic Condenser 1.40 N.A. is a divisible three-element lens system, with spherical correction carried to the highest degree for all zones; with the upper lens removed, the N.A. is 0.60; with two lenses removed, the remaining one has 0.40 N.A.

The Achromatic Condenser is similar to an oil-immersion objective in its construction, being chromatically and spherically corrected. With this condenser it is possible to image into the microscope field a diaphragm placed at the light source and, thereby, eliminate undesirable objects in the field of view without introducing objectionable color bands which cloud the field.

This condenser has a N.A. of 1.40 and a focal length of 9.2 mm. It will work through a slide of 1.2 mm. thickness. The top portion may be removed when using low power objectives of 0.3 N.A. or less.

The Triple Lens Condenser is an Abbé condenser with a supplementary lens below the iris diaphragm for quick interchange to low power observation. This additional lens obviates the need for removing the top element when changing to low power work.

Dark field with the paraboloid condenser. The paraboloid condenser is used for the examination of very minute particles or organisms. The condenser is designed for use with high power oil-immersion objectives and intense illumination is required for best results.

The correct place for the object is the point where the light comes to a focus. Light passes through this point under an angle equal to numerical apertures from 1.24 to 1.33. The objectives used with this condenser must have a numerical aperture less than 1.24. Best results will be secured if the N.A. of the objective does not exceed 1.00.

In using the condenser, it is necessary that there be cedar oil or glycerin between the condenser and the slide, since the angle of incidence of light on the surface of the slide is beyond the critical angle. Thus, if air bounds the surface, no light will pass from the condenser. Furthermore, it is necessary that the specimen to be illuminated be mounted in liquid or cement and protected with a cover glass.

The objective used may be either dry or immersion as long as the numerical aperture is not as great as the numerical aperture of the condenser.

For best results, the glass slide upon which the specimen is mounted should not be thicker than 1.4 mm.

Diaphragms

In addition to the lenses of the optical system of the microscope, the various diaphragms are important for they regulate the apertures of the various lenses and the size of their field image.

Aperture diaphragms are placed so as to limit the cone of rays which is transmitted by their respective lenses. Reducing the opening of the aperture diaphragm reduces the illumination uniformly over the entire field and no decrease in the size of the illuminated field results.

Field diaphragms are placed so as to be imaged coincidentally with the image of the object and to limit the area visible. Reducing the opening of the field diaphragm decreases the intensity of the light uniformly over the entire aperture. The full opening is utilized by the cone of rays which converge in each image point but less light is required since the area of the illuminated field is decreased. The brightness of the illuminated portion is not reduced though the area is smaller.

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CHAPTER II

ILLUMINATION

In addition to good optical equipment and nicely working movements, there is nothing more important for good results than proper illumination of correctly prepared objects. The illumination governs resolution, the visibility of fine details, and the naturalness of the object as a whole. Proper illumination technique is necessary in critical microscopy, and incorrect methods of illumination may neutralize the value of even the best optical equipment.

The different methods of illumination can be divided into two main groups: illumination of transparent objects and illumination of opaque objects. Each of these two main divisions of illumination is again divided into different methods of application.

ILLUMINATION OF TRANSPARENT OBJECTS

Illumination by the mirror alone can be used only for relatively low magnifications. If a plane mirror is used, only the direction of the light is changed (Figure 14).

The concave mirror may be used for illumination by transmitted light without a condenser. It serves chiefly to converge the light upon the object and to give brighter illumination over a smaller field (Figure 15). The concave mirror can also be used for simple dark field illumination (Figure 16).

AXIAL TRANSMITTED LIGHT (FIGURE 17)

If the condenser is not centered with respect to the axis of the microscope, it may be impossible to obtain strictly axial illumination (AA'). Strictly axial illumination is valuable in the examination of objects of low visibility which give faintly outlined refraction images. It is also useful in the interpretation of shading in terms of structure, in the observation of polarization colors, in testing refractive index, and for micrometric measurements.

CONVERGENT TRANSMITTED LIGHT

The degree of convergency is regulated by the iris diaphragm or by adjusting the height of the condenser. These manipulations simultaneously affect the brilliance of the illumination, as well as its convergency.

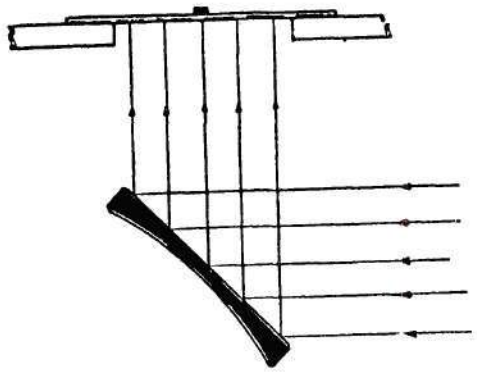

Figure 14. Illuminating Object with Plane Mirror

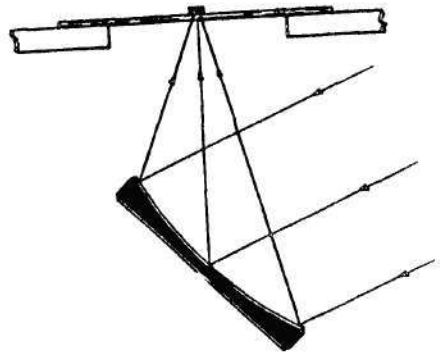

Figure 15. Illuminating Object with Concave Mirror

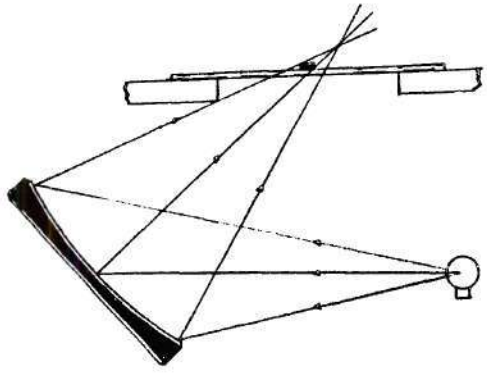

Figure 16. Simple Dark-Field Illumination

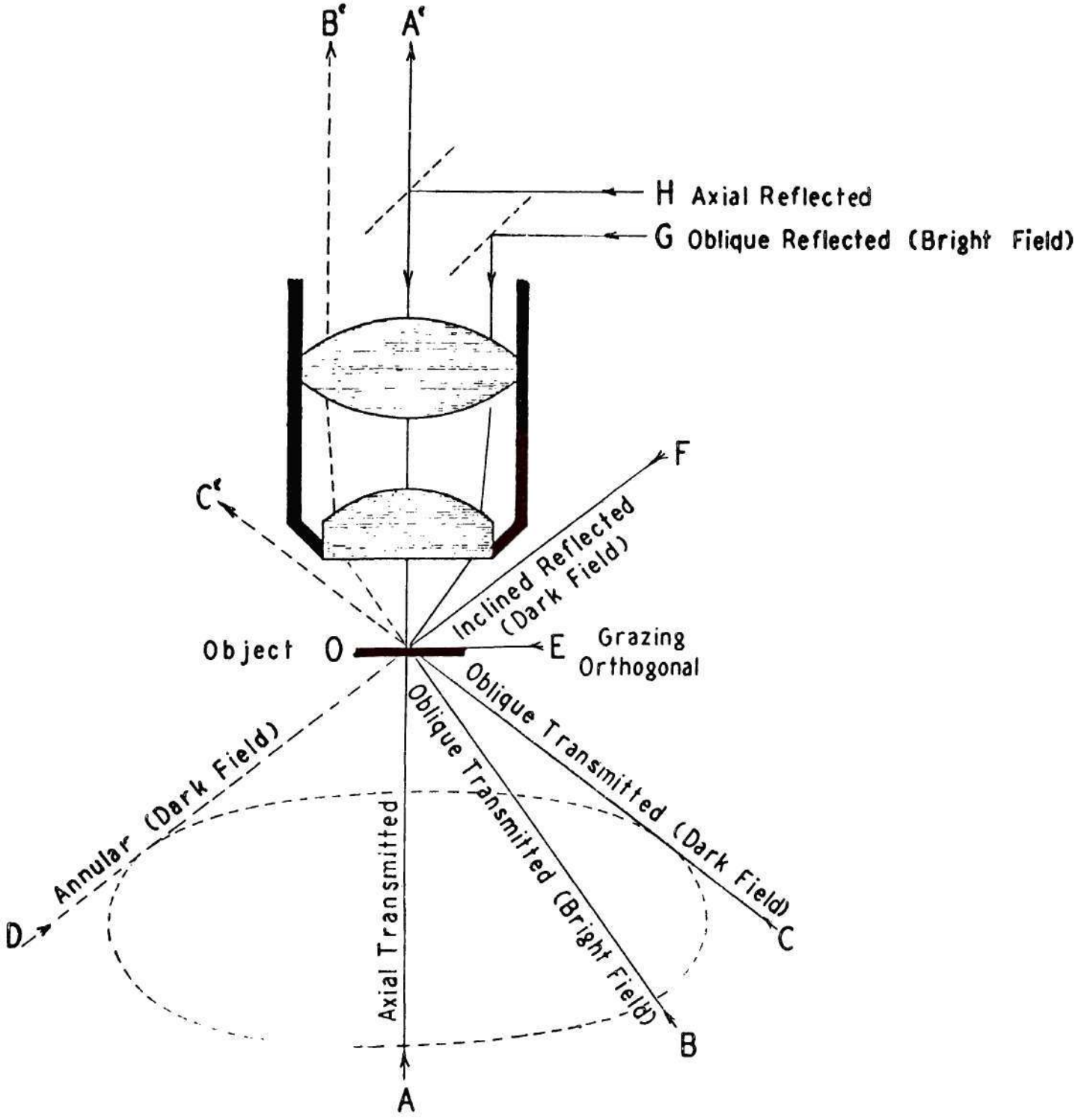

Figure 17. Relationship of Various Types of Illumination

(Chamot Chemical Microscopy)

Condensers of long focal length do not require as perfect centering as those of higher aperture on account of the large field which is illuminated.

Symmetrical convergent illumination is employed in practically all critical work at magnifications greater than 100 and is highly advantageous at lower magnifications.

The exact control of intensity and the degree of convergency is of great aid in the study of internal structures of transparent substances, such as textile and paper fibers, crystals, and other microscopic objects. Careful regulation of the diaphragm opening will reveal numerous features which could otherwise be overlooked.

OBLIQUE TRANSMITTED LIGHT (FIGURE 17, BB')

Oblique transmitted light should always be used as a supplement to symmetrical or axial illumination.

Highly oblique (dark field) illumination may be obtained if the condenser has a distinctly greater aperture than the objective (CC').

Emulsions, crystals, starches, and any object possessing superposed structures may be very realistically observed and photographed, so that their three dimensional characteristics are apparent.

ANNULAR OBLIQUE ILLUMINATION

Annular oblique illumination is obtained by means of a hollow cone of rays, oblique in all azimuths and converging upon the object. The cone of rays must be focused on the object, it must be symmetrical, and it must be centered. In the case of examination by transmitted light, the obliquity is such that no direct rays are included within the angular aperture of the objective, and dark field illumination results (Figure 17, DO).

Annular oblique dark field illumination is one of the most useful types of illumination when properly manipulated and can be divided into three forms: dark field with ordinary condensers and wheel stops or Rheinberg filters (Figure 18), dark field with paraboloid condenser (Figure 19), and, finally, dark field with the cardioid condenser (Figure 20), which is not only the most efficient dark field illumination but also the most difficult.

DARK FIELD ILLUMINATION WITH WHEEL STOPS (FIGURE 18)

The operations necessary to produce dark field illumination for low power work are as follows: Place the specimen on the stage of the microscope and focus with a low power objective using ordinary illumination. The condenser

and the mirror will then be centered. The wheel stop is placed in the carrier and the iris diaphragm is opened full. Raise and lower the condenser until the object is seen brightly illuminated on a black background. If the background appears gray or partly illuminated use a larger stop. If it is found that, despite raising or lowering the condenser, the object is imperfectly illuminated, a smaller stop must be used.

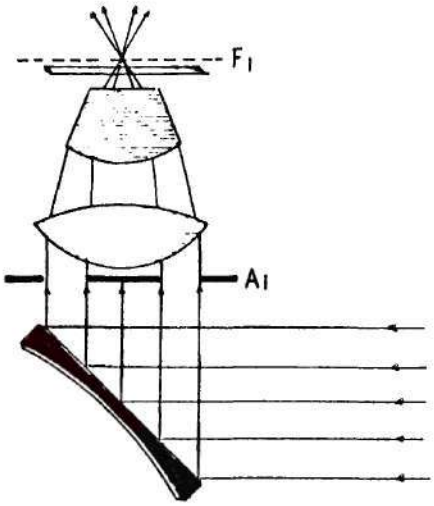

Figure 18. Illumination with Central Stop

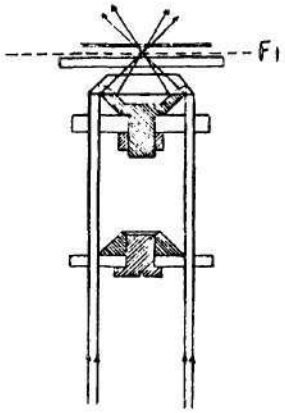

Figure 19. Paraboloid Condenser

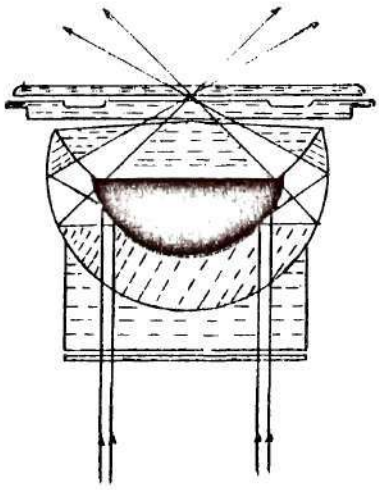

Figure 20. Path of Light Through the Cardioid Condenser

Differential color illumination is obtained by the use of the Rheinberg filters and is similar in principle to dark field illumination. The disk functions as a central stop to control the color of the field. The central color determines the color of the field and the annular screen determines the color of the object.

PARABOLOID OR DARK GROUND ILLUMINATOR (FIGURE 19)

Dark ground illuminators require somewhat greater care in adjustment than the ordinary condenser. These points are of importance:

1. Contact liquid between condenser and slide.
2. Object must not be mounted dry.
3. Numerical aperture of the objective should be at least 0.10 smaller than that of the condenser.
4. Perfect centering.
5. Focus light in plane of object.
6. Slide thinner than working distance of condenser.
7. Preparation as thin as possible. Colloidal suspensions must not be too concentrated.

THE REFRACTIVE INDEX

Using a low power objective and axial transmitted light the rays emerging from a spherical fragment of the substance, if originally parallel, will become divergent whenever the refractive index of the substance is less than that of the medium. Consequently, the appearance of the object in correct focus will apparently expand when the objective of the microscope is raised, and the object is surrounded by a bright ring of light. The bright ring expands from the center of the substance. If the objective is lowered below the exact focus, the opposite takes place.

If the substance is illuminated with oblique transmitted light (B-O, Figure 17) the part of the field on the same side as the condenser diaphragm opening will become dark and the opposite side will become bright. That is, the object will appear dark on the side near the light half of the field and light on the opposite side.

Parallel rays passing through the substance when the refractive index of the substance is greater than that of the medium will tend to become convergent, and hence, upon raising the objective from the position of correct focus, the bright ring (which in this case is seen within the dark counter bands) will apparently contract and move toward the center of the substance. When illuminated with a pencil of oblique light the opposite effect to the one already described will be observed and the object will be dark on the side nearest the dark part of the field.

To determine the refractive index of a solid it is placed on a slide and covered with a drop of a liquid which is believed to have about the same value. The mount is then focused with a 32 or 16-mm. objective and the rings noted; the objective is raised out of focus and the movement of the bright rings watched; finally, the object is illuminated with a pencil of oblique transmitted light and it is observed which side of the substance darkens.

These observations, which require only a few seconds, allow one to ascertain whether the liquid is of higher or lower refractive index than the solid. The procedure is repeated using a liquid deemed nearer the true refractive index of the object until the value is found. With a little practice $\underline{n}$ may be found easily within $\pm$ 0.007.

Monochromatic light, obtained by using a color filter, may be used if greater accuracy is required.

Accurate results are obtained only after the immersion liquid has penetrated the fiber completely.

The refractive index of a liquid or mixture of liquids can be determined easily in an Abbé refractometer.

The refractive indices of suitable liquids for determining those of fibers and other substances are given in the following table. Most of these values were taken from a recent edition of the Handbook of Chemistry and Physics published by the Chemical Rubber Publishing Co.

TABLE II

THE REFRACTIVE INDICES OF SOME IMPORTANT MOUNTING MEDIA

Alphabetical Listing		Listing According to Ascending Values of n	
n	Name	n	Name
1.359	Acetone	1.000294	Air
1.000294	Air	1.331	Methyl alcohol
1.410	Amyl alcohol	1.333	Water
1.562	Anethole	1.359	Acetone
1.586	Aniline	1.362	Ethyl alcohol
1.546	Anise oil	1.375	n-Hexane
1.518	Anisole	1.389	n-Heptane
1.501	Benzene	1.397	Glycerol and water (1:1)
1.540	Benzyl alcohol	1.399	Butyl alcohol
1.465	Bergamot oil	1.400	Ethylene glycol monoethyl ether ("Cellosolve")
1.560	Bromobenzene	1.405	Paraldehyde
1.659	α-Bromonaphthalene	1.408	Isoamyl alcohol
1.598	Bromoform	1.410	Amyl alcohol
1.399	Butyl alcohol	1.423	Dioxane
1.465	Cajeput oil	1.430	Triacetin
1.521	Canada balsam	1.446	Chloroform
1.477	Caraway oil	1.446	Kerosene
1.630	Carbon disulfide	1.446	Trimethylene chloride
1.463	Carbon tetrachloride	1.452	Paraffin oil, light
1.490	Castor oil	1.458	Cineole

TABLE II (Continued)

THE REFRACTIVE INDICES OF SOME IMPORTANT MOUNTING MEDIA

Alphabetical Listing

n	Name
1.510	Cedar oil
1.446	Chloroform
1.458	Cineole
1.584	Cinnamon oil
1.540	Clarite
1.570	Clarite X
1.533	Clove oil
1.545	Colophony
1.535	Creosol
1.517	Crown glass
1.552	o-Dichlorobenzene
1.423	Dioxane
1.362	Ethyl alcohol
1.538	Ethylene bromide
1.400	Ethylene glycol monoethyl ether ("Cellosolve")
1.535	Euparal
1.529	Fennel oil
1.530ca.	Gelatin
1.473	Glycerol
1.397	Glycerol and water (1:1)
1.521	Gum dammar
1.389	n-Heptane

Listing According to Ascending Values of n

n	Name
1.463	Carbon tetrachloride
1.463	Palm oil
1.465	Bergamot oil
1.465	Cajeput oil
1.468	Olive oil
1.470	Venice turpentine
1.472	Turpentine
1.473	Glycerol
1.477	Caraway oil
1.480	Linseed oil
1.481	Nujol
1.483	Terpineol
1.490	Castor oil
1.496	p-Xylene
1.497	Toluene
1.501	Benzene
1.505	Myrrh oil
1.508	Parlodion
1.508	Phenetole
1.510	Cedar oil

TABLE II (Continued)

THE REFRACTIVE INDICES OF SOME IMPORTANT MOUNTING MEDIA

Alphabetical Listing		Listing According to Ascending Values of n	
n	Name	n	Name
1.375	n-Hexane	1.513	Trimethylene bromide
1.408	Isoamyl alcohol	1.517	Crown glass
1.568	Isoeugenol	1.518	Anisole
1.446	Kerosene	1.520	Sandalwood oil
1.480	Linseed oil	1.521	Canada balsam
1.331	Methyl alcohol	1.521	Gum dammar
1.738	Methylene iodide	1.529	Fennel oil
1.780	Methylene iodide saturated with sulfur	1.529	Mustard oil
1.529	Mustard oil	1.530ca.	Gelatin
1.505	Myrrh oil	1.533	Clove oil
1.659	Naphthalene, 1-bromo	1.535	Creosol
1.553	Nitrobenzene	1.535	Euparal
1.481	Nujol	1.537	Wintergreen oil
1.468	Olive oil	1.538	Ethylene bromide
1.463	Palm oil	1.540	Benzyl alcohol
1.452	Paraffin oil, light	1.540	Clarite
1.405	Paraldehyde	1.542	Safrole
1.508	Parlodion	1.545	Colophony
1.508	Phenetole	1.546	Anise oil

TABLE II (Continued)

THE REFRACTIVE INDICES OF SOME IMPORTANT MOUNTING MEDIA

Alphabetical Listing

n	Name
2.060	Phosphorus (yellow) sulfur and methylene iodide (8:1:1 by weight)
1.622	Quinoline
1.542	Safrole
1.520	Sandalwood oil
1.62+	Styrax
1.483	Terpineol
1.546	Tetralin
1.640	Tolu balsam, solid
1.497	Toluene
1.573	o-Toluidine
1.430	Triacetin
1.513	Trimethylene bromide
1.446	Trimethylene chloride
1.472	Turpentine
1.470	Venice turpentine
1.333	Water
1.537	Wintergreen oil

Listing According to Ascending Values of n

n	Name
1.546	Tetralin
1.552	o-Dichlorobenzene
1.553	Nitrobenzene
1.560	Bromobenzene
1.562	Anethole
1.568	Isoeugenol
1.570	Clarite X
1.573	o-Toluidine
1.584	Cinnamon oil
1.586	Aniline
1.598	Bromoform
1.62+	Styrax
1.622	Quinoline
1.630	Carbon disulfide
1.640	Tolu balsam, solid
1.659	α-Bromonaphthalene
1.659	Naphthalene, 1-bromo
1.738	Methylene iodide
1.780	Methylene iodide saturated with sulfur

TABLE II (Continued)

THE REFRACTIVE INDICES OF SOME IMPORTANT MOUNTING MEDIA

Alphabetical Listing		Listing According to Ascending Values of n	
n	Name	n	Name
1.496	p-Xylene	2.060	Phosphorus (yellow), sulfur and methylene iodide (8:1:1 by weight)

The refractive index of many of these materials is somewhat variable, especially in the case of oils and resins.

ILLUMINATION OF OPAQUE OBJECTS

Although the reflecting property of opaque objects is necessarily the basis of their microscopic visibility, the methods of examination by reflected light are also frequently applicable to relatively transparent materials.

Practice in the illumination and study of transparent objects is a valuable preliminary training for work with reflected light.

INTERPRETATION OF APPEARANCES

The interpretation of appearances by reflected light depends almost entirely upon the application of the laws of reflection, in particular, that the angle of reflection of light from a surface is equal to the angle of incidence. The pattern of light and dark, which constitutes the reflection image seen in the microscope, will appear bright if oriented so as to reflect light within the angular aperture of the objective and dark otherwise.

A plane mirror surface (such as that of a polished unetched metal) lying on a plane normal to the axis of the microscope and illuminated by an inclined beam will reflect light specularly. If the angle of incidence is greater than half the angular aperture of the objective, the angle of reflection will be such that no directly reflected rays can be included, and no light will pass through the microscope. Consequently, the surface will appear dark, and, the more perfectly it reflects, the darker it will be. If the angle of incidence is decreased to less than half the angular aperture of the objective, the illumination beam will be reflected in a direction which comes within the angular aperture of the objective, and the surface will appear bright.

If the surface of the object illuminated by the inclined beam is roughened or scratched, or if it is irregular in places, the irregular areas will reflect less specularly than the smooth portion and will scatter light in many different directions. As a consequence they will appear light with inclined illumination from outside the angular aperture of the objective and dark if the incident light comes within this aperture.

METHODS OF ILLUMINATION BY REFLECTED LIGHT

The examination of opaque surfaces under the microscope is an operation applicable to many subjects in the paper mill laboratory. The following are requisite for this purpose:

1. A concentrated source of artificial light such as a small arc lamp, a "Pointolite" lamp, or a small metal filament lamp of the projection type.

2. Optical means for the projection of the light rays obliquely to the surface under examination.

3. For critical comparative work, means for determining the exact angle of incidence is also a necessity.

APPLICATIONS OF OPAQUE ILLUMINATION

Opaque illumination by oblique light finds many applications in paper mill work. The most important of these is the examination of surfaces of printing paper in both the printed and unprinted conditions. Many papers having a high degree of finish, as indicated by the glarimeter method of analysis, show an inferior printing surface. Closeness of surface is much more important than a high gloss on the top levels of a broken surface.

The distribution of ink in printing is a subject of equal importance which may be observed by this method of illumination. The wire marks of the sheet, the presence of air bubbles in coated papers, and the physical effect of calendering are easily observed and studied in detail. Under high magnification the distribution of sizing and loading materials may be observed quite clearly.

In addition to the examination of paper this method of illumination is valuable for the observation of wear in wire and felts.

LOW POWER ILLUMINATION

The simple methods in use 50 to 75 years ago for the illumination of opaque surfaces under low powers of the microscope were quite efficient and may be applied by the use of very simple and inexpensive apparatus--for

example, the bull's-eye condenser (Figure 21), side reflectors (Figure 22), Amici prisms (Figure 23), and the Lieberkuhn reflector (Figure 24) (1).

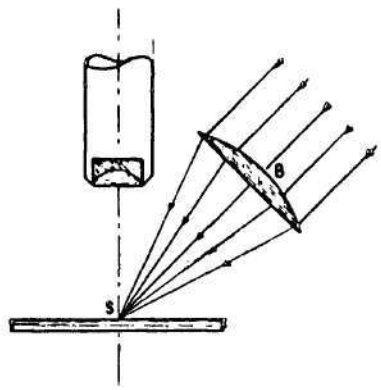

Figure 21. Simplest Method of Using Bull's-eye Condenser

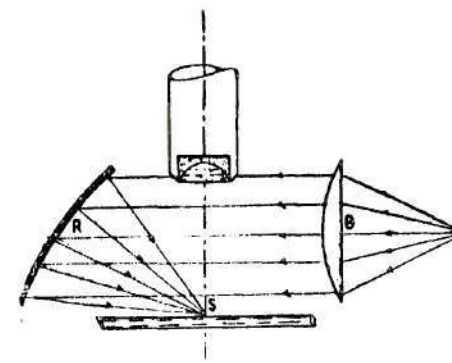

Figure 22. Use of Paraboloid Metal Reflector

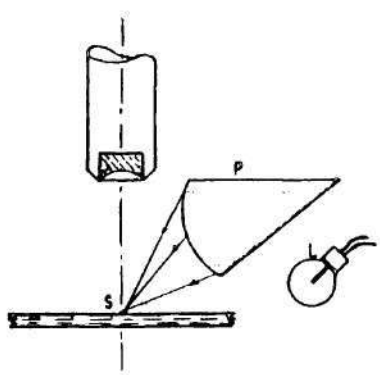

Figure 23. Use of Amici Prism

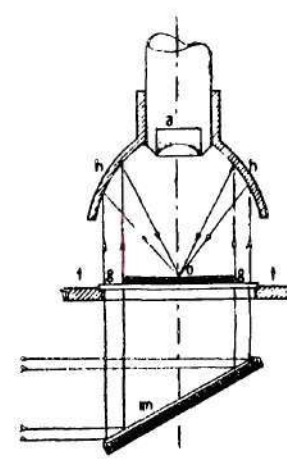

Figure 24. Old-fashioned "Lieberkuhn" Illuminator

More modern equipment includes the Beck ring illuminator (Figure 25), the Hauser illuminator (Figure 26), the Silvermann illuminator, the Bausch and Lomb surface illuminator, the Bausch and Lomb papermaker's microscope, Beck illuminator (Figure 27) (2), and Graff's variable angle surface illuminator (Figure 28) (3).

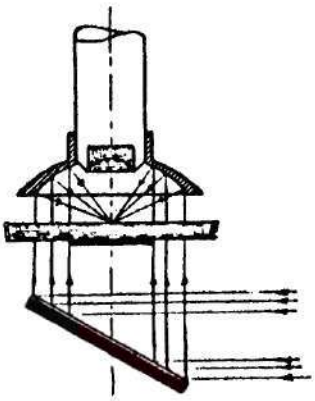

Figure 25. Beck Ring Illuminator

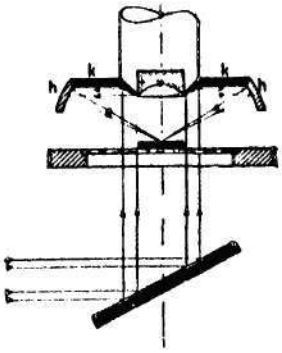

Figure 26. The Hauser Illuminator

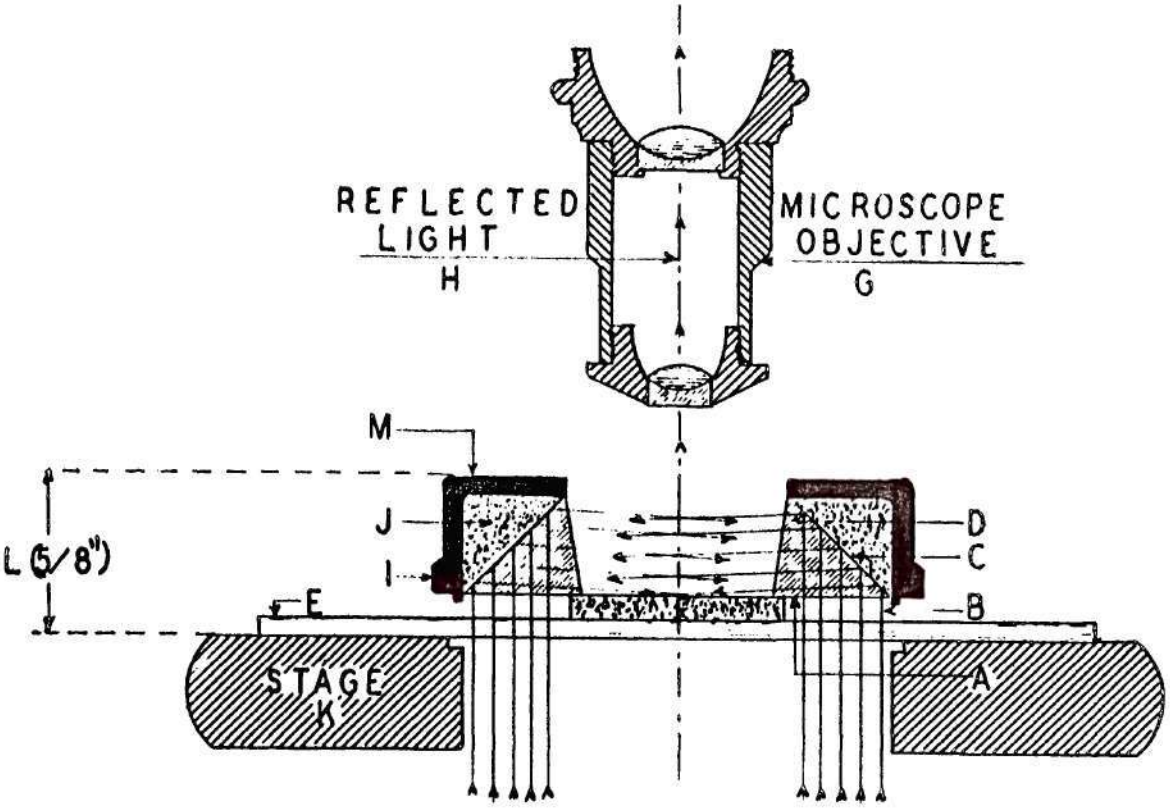

Figure 27. Cross Section of Beck's Illuminator Mounted on Stage

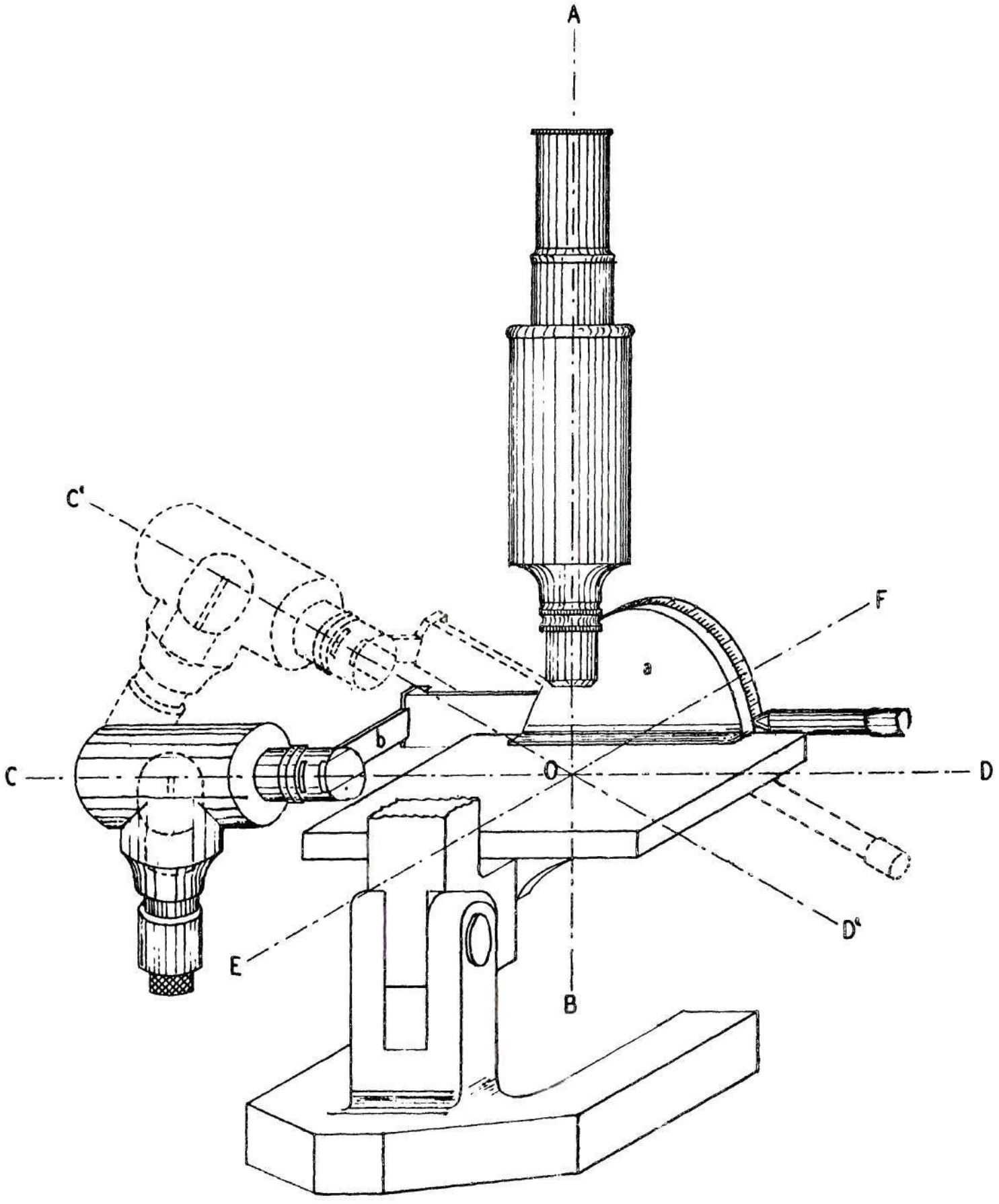

Figure 28. The Variable Angle Surface Illuminator

The variable angle surface illuminator (Figure 28) is a microscope illuminator for photographic and visual inspection of the surface texture of materials, based upon the principles that, if the optical axis of the microscope, the optical axis of the illuminant, and the axis of rotation of the illuminant (perpendicular to the axis of illumination) intersect at the same point in the plane of observation, regardless of the angle of incidence of the illuminant, and the surface of the material to be examined lies in the optical plane with its machine direction parallel to the axis of rotation of the illuminant, visual comparison of such surface textures can be made by photographing comparable materials at a given angle of incidence.

When a sample of paper or any other material is placed on the microscope (Figure 29) with the illuminator set in such a position that the machine direction of the sample is parallel to the axis of rotation of the illuminant, and its top surface lies in the plane of the intersection of the axis, then the surface texture when illuminated with a grazing beam of light or a light beam at a high angle of incidence will present a pattern of lights and shadows (Figure 30). The length of these shadows can be used to determine the depth of the depressions by measuring the length of the shadows and dividing it by the reciprocal of the tangent of the angle of incidence. These shadows will gradually disappear as the angle of incidence of the light beam is decreased.

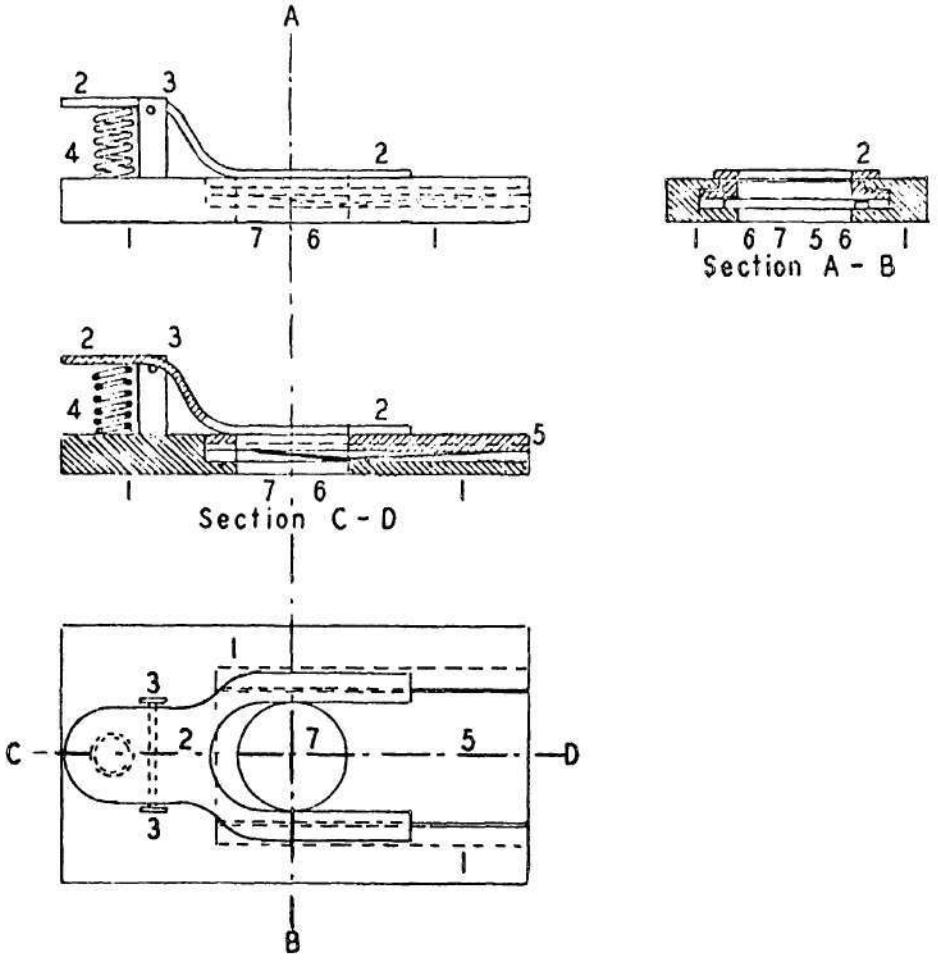

Figure 29. Sample Holder for the Variable Angle Surface Illuminator

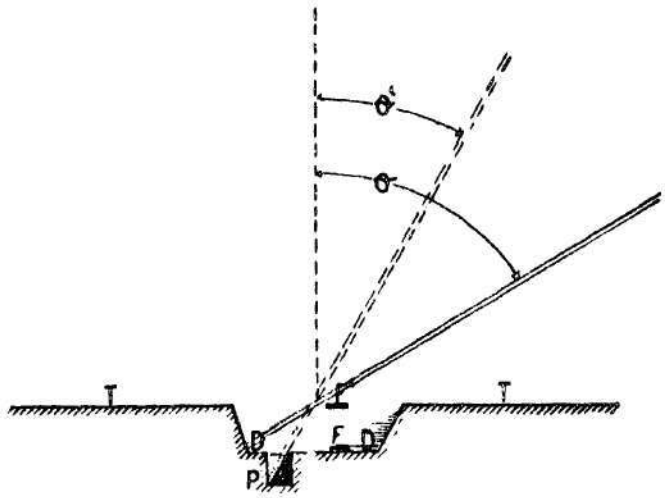

Figure 30. Highlights and Shadows in Relation to the Angle of Incidence of the Light Beam

AXIAL ILLUMINATION AND VERTICAL ILLUMINATORS

Axial illumination may be obtained by placing a reflector between the objective and the specimen, so as to send the light practically parallel to the axis of the microscope and normal to the surface under examination (Figure 31).

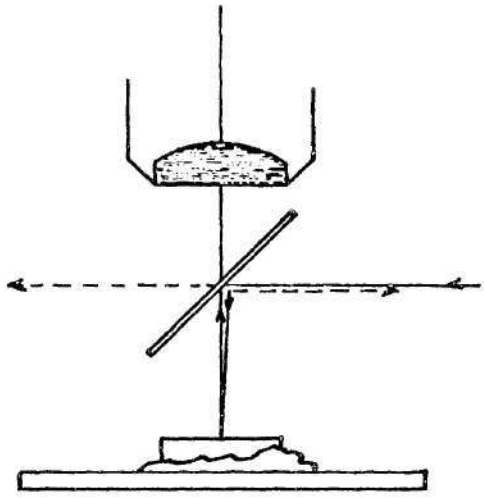

Figure 31. Axial Illumination with Cover Glass Placed Between Objective and Object

External reflectors, of course, cannot be used with objectives of short working distance. Inclined illumination may be obtained by moving the transparent reflector somewhat to one side of the axis of the microscope, altering its slope or the position of the light source if necessary. This is not necessary, however, and a direct beam of the light source is commonly employed.

Vertical illuminators consist of a mounting containing some sort of reflector which sends light through the objective to the specimen but does not seriously obstruct the passage of the image. The objective functions as a condenser to illuminate the object and also forms a real image of it in the microscope. Short focal lengths and working distances set no limitation on the use of vertical illuminators.

The main types of such illuminators are the transparent reflector vertical illuminator (Figure 32), the prism vertical illuminator (Figure 33), and the mirror vertical illuminator (Figure 34).

Short mounted objectives should always be used for this type of illumination.

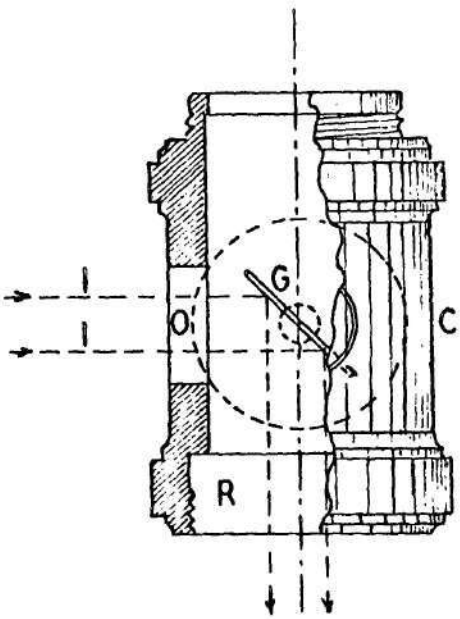

Figure 32. Transparent Reflector Vertical Illumination

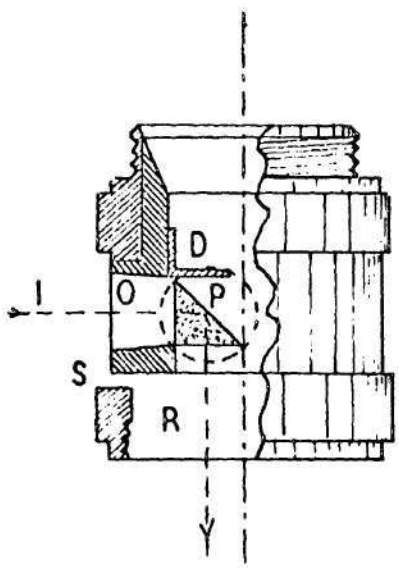

Figure 33. Prism Vertical Illuminator

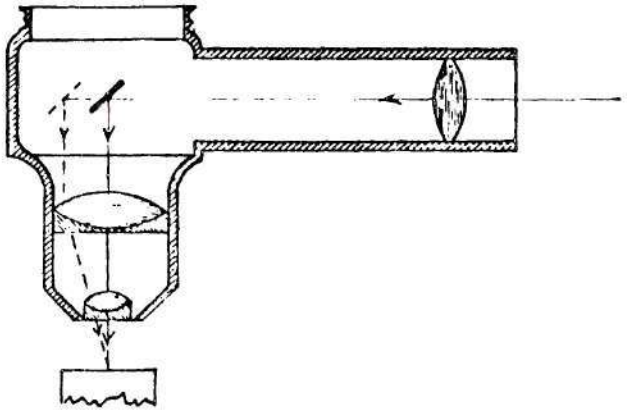

Figure 34. Mirror Vertical Illuminator

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CHAPTER III

EXAMINATION IN POLARIZED LIGHT

Although polarized light at first seems to be complicated and of little practical use, its role in microscopic studies offers many advantages and can supply much information, both for research and routine examinations, that cannot be obtained any other way.

POLARIZED LIGHT (15)

The microscopist can use one of several methods to produce plane-polarized light:

1. Reflection from a polished surface of a refracting material at the appropriate angle.

2. Transmission through a Nicol prism or modification of this form of prism.

3. Transmission through a plate of tourmaline cut parallel to the optic axis.

4. Transmission through plates of Polaroid (a compound of iodine and quinine sulfate crystallizing in the form of thin, flat plates).

Arrangement of two polarizers in optical train so oriented that the vibration directions of light transmitted by them are parallel and will allow light to pass through the second polarizer, or analyzer. If, however, one of them is rotated so that its plane of vibration is now at right angles to that of the other, no light will pass. The former condition is commonly spoken of as "parallel Nicols" and the latter as "crossed Nicols" (Figure 35).

Various materials behave differently with respect to the transmission of light. To demonstrate this phenomenon certain specimens such as a sheet of glass, quartz, cellophane or selenite are placed between crossed Nicols and rotated in their own plane perpendicular to the direction of vision. The glass (provided it is homogeneous and free from strain) will have no effect on the dark field given by the crossed Nicols; the quartz, however, will appear alternately dark and light at each 45° of rotation; the cellophane (if thin and of uniform thickness) will appear colored; and the thin plate of selenite (which usually cleaves in layers of varying thickness) will give a multicolored appearance when placed at 45° between the crossed Nicols.

Figure 35. Control of Light by Superimposed Polarizers

The well-known property of double refraction is responsible for the effects seen with the last three materials. This may also be demonstrated by Huygens' early experiments, in which two images will be seen instead of one when a rhomb of Iceland spar (calcite) is placed over some print. In a second experiment an illuminated slit may be viewed through a polished crystal of quartz when two spectra will be evident (Figure 36). It can be shown that each beam is plane-polarized by inserting an analyzer in each of the beams but that their vibration directions are mutually perpendicular. Furthermore, the two beams are deviated by different amounts, the refractive index of the material for the two beams must be different, hence the two beams travel with different velocities through the medium. Thus, if a beam of light enters a birefracting material it is split into two beams, vibrating at right angles to each other and traveling with different speeds through the material.

Many polarization phenomena can be explained on the basis of such behavior. For example, the color of a thin plane parallel plate of some birefracting substance when placed at 45° between crossed Nicols and illuminated by white light is due to the fact that the two beams traveling with different velocities through the plate get out of step or out of phase by half a wavelength of one color of the spectrum; hence this color is eliminated by interference, and the final appearance of the spectrum is white minus the portion eliminated. For a more adequate explanation, however, a close study of polarization phenomena generally is required.

OPTICALLY ISOTROPIC AND ANISOTROPIC MATERIALS

There are two classes of substances when considered in relation to their optical symmetry: isotropic and anisotropic. A material which has no effect on polarized light, transmitting it with equal facility in any position, is termed isotropic. Solids of this type are rather rare, and even these, if put into condition of strain, become anisotropic, as may be readily demonstrated by straining a glass thread under the polarization microscope. The paucity of this class of substance makes the negative information of value.

All other substances are anisotropic. The phenomena of double refraction, or birefringence, is one of the manifestations of anisotropy. Double refraction means the possession of more than one refractive index, depending on which direction the light travels through the substance and the plane of polarization of the light used to measure the index. A crystal possesses one, two, or three refractive indices depending on the class of symmetry to which it belongs. Those possessing either two or three refractive indices are anisotropic, and are those of which fibers are built.

The cellulose micelle is the fiber crystal unit which has been most fully investigated. According to Preston (17) only two indices have been measured and the third index, which cellulose must possess if the crystal structure deduced from x-ray spectroscopy is correct, has not been definitely proved to exist. However, Frey-Wyssling (6, 7) states that the two indices perpendicular to the fiber axis differ only slightly.

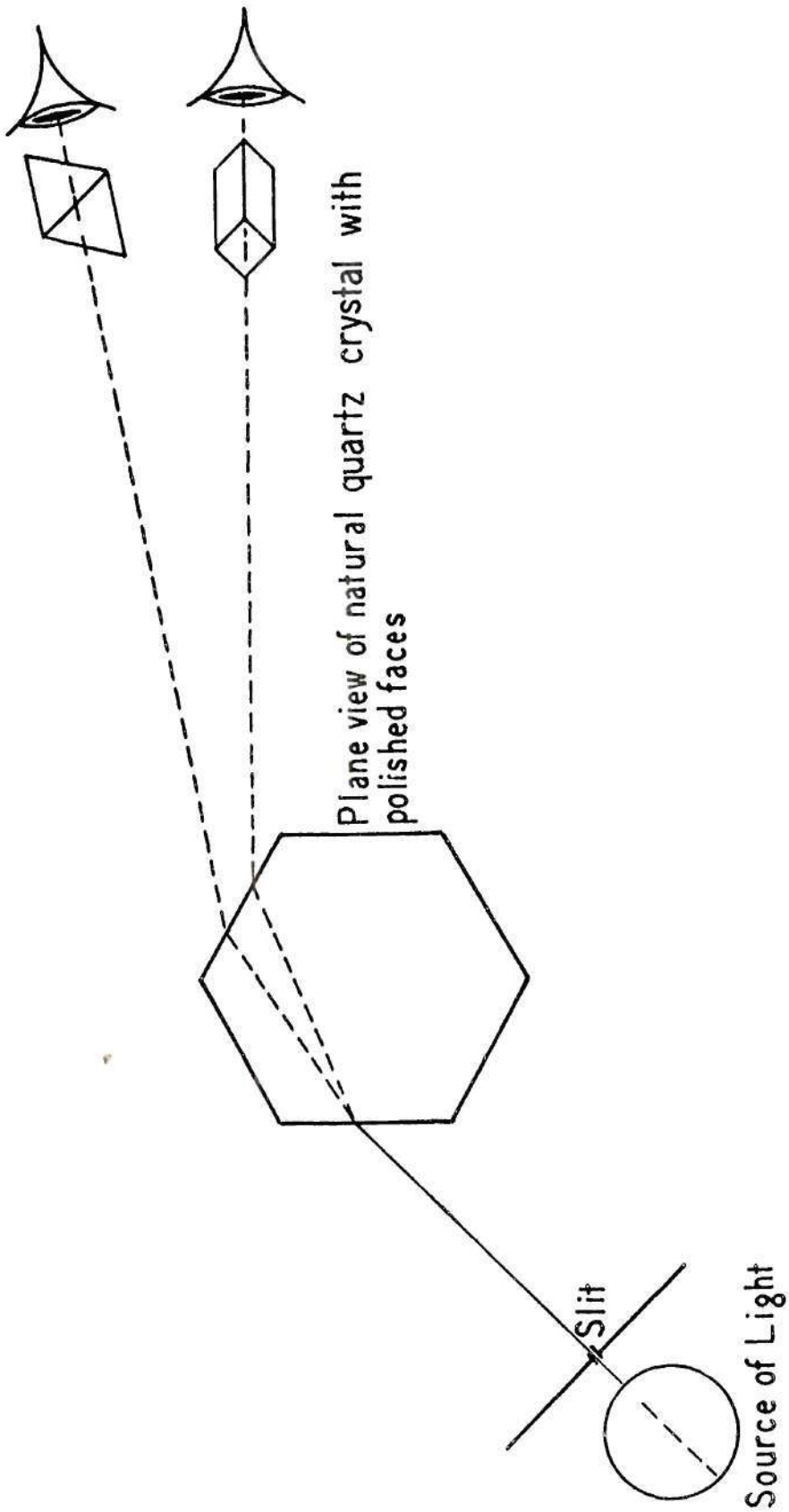

Figure 36. Formation of Two Spectra by Refraction Through a Polished Crystal of Quartz

Perhaps it should be considered that with either random or concentric lateral orientation the object would be optically uniaxial with two main refractive indices, one in the direction of the chain and one perpendicular to it (13). All fibers investigated appear to possess the same symmetry as a positive or negative uniaxial crystal, in which the axis of symmetry lies parallel to the fiber axis. That is, a fiber possesses a different refractive index for light of which the vibrations are parallel to the fiber axis than it does for light which vibrates perpendicular to the fiber axis; but the refractive index is the same no matter in which direction perpendicular to the fiber axis the light vibrates.

CAUSE OF DOUBLE REFRACTION

Three factors operate separately or together to produce double refraction: strain, intrinsic anisotropy, and form anisotropy.

STRAIN

Solid bodies which are isotropic become anisotropic if they are stretched or compressed. An isotropic body then behaves like an uniaxial crystal whose principal axis lies parallel to the direction of the applied areas. In compression the sign of the double refraction is negative (the larger refractive index is that component in which the light vibrates perpendicular to the principal axis); in extension the sign of the double refraction is reversed. Stressing beyond the elastic limit may cause the micelles of gels and plastic bodies to become oriented in the direction of the strain. For example, imperfectly oriented cellulose fibers have their double refraction increased when stressed in a plastic state. Removal of the stress eliminates the double refraction but the effect of orientation produced by strain remains.

INTRINSIC ANISOTROPY

Intrinsic anisotropy is that of the crystal unit of the fiber irrespective of how the units are grouped to form the fiber. Even though all the units are perfectly oriented in the fiber it does not have the same refractive index as the fiber unit unless the fiber is mounted in a liquid whose refractive index is the mean of the two indices of the crystal unit. The intrinsic refractive index of lignin has been shown to be 1.62 by finding that a medium of that refractive index reduced the double refraction of lignin to zero (5).

The anisotropy of fibers built of perfectly oriented micelles is very high when all the micelles are oriented with their long axis parallel to that of the fiber but when the axis of consecutive micelles is arranged along a spiral the anisotropy of the fiber will be lower than with the parallel arrangement (6).

FORM ANISOTROPY

Under certain conditions anisotropy may be caused by the arrangement and shapes of the micelles, even though these may be isotropic. Arrangement of rodlets or disks in an orderly fashion with their axes parallel could produce double refraction in a body if the substance between the structural units had a different refractive index than that of the units. All natural and synthetic fibers show rod-form double refraction in addition to their intrinsic double refraction (17).

THE MEASUREMENT OF DOUBLE REFRACTION (17)

The double refraction of fibers may be measured in two ways. The more straightforward is to measure the two refractive indices by the Becke method, with the plane of polarization first parallel (n_α) and then perpendicular (n_γ) to the fiber axis at a particular wavelength. The difference ($n_\gamma - n_\alpha$) at the same wavelength is the double refraction.

The alternate method is to find the amount of retardation of the ordinary ray behind the extraordinary ray after passing through the fiber. The relation is $\rho = \frac{\delta\lambda}{d}(n_\gamma - n_\alpha)$, where ρ is the double refraction, δ is the retardation, d is the thickness of the fiber, and λ is the wavelength, all expressed in micrometers. Accurate determination of the thickness is difficult. Retardation, on the other hand, is measured very easily. The fiber must be arranged in the position which gives the maximum retardation, that is, must lie at 45° to the planes of the crossed Nicols between which it is placed. The retardation is measured with a compensator calibrated to read directly. The compensator is used to just neutralize the retardation due to the fiber. Thus, if the ray of light vibrating parallel to the fiber axis lags behind the ray of light vibrating perpendicular to the fiber axis by an amount "δ," then the compensator is adjusting until the second ray is retarded by this amount "δ" behind the first ray. The fiber then appears to be isotropic (black between crossed Nicols in the 45° position).

The Becke method is independent of the form-anisotropy of the fiber, but the compensator method is only partially independent, and then only when the refractive index of the immersion liquid lies in the region between the two indices of the fiber. However, the error in this case is less than one percent of the double refraction, and is not as great as that which is likely to arise in the measurement of fiber thickness.

RETARDATION (17)

When the fiber lies at 45° to the planes of the two crossed Nicols, the plane-polarized light reaching the fiber is separated into two beams polarized at right angles to each other, and at 45° to the incident beam. If the intensity of the incident beam is I_o, the intensity of the two beams in the fiber are $I_o \cdot \cos^2 45°$ and $I_o \cdot \sin^2 45°$. But $\sin 45° = \cos 45°$, so the intensities of the two component beams are equal; at any other angle the intensities are no longer the same. The beams travel through the fiber with velocities inversely proportional to the refractive indices. As these velocities differ, the light of the two component beams will no longer be in phase when it emerges from the fiber. Passing through the analyzing prism permits only the portion of the two beams resolved in the plane of the analyzer to be transmitted. Since this light is now vibrating in the same plane, and is no longer in phase, it will show interference.

When monochromatic light is used, this interference manifests itself as total extinction of the light when one of the component beams in the fiber is retarded an odd number of half wavelengths behind the other. Conversely, when one beam is retarded an exact number of whole wavelengths behind the other, the two will reinforce each other and the fiber will appear at its brightest. In between these two states the fiber will appear of intermediate brightness.

The amount one beam is retarded depends on the two refractive indices and the fiber thickness. Fibers are generally thickest in the center and gradually become thinner toward the edge. Consequently, the interference effect goes through a number of extinctions and reinforcements if the fiber is thick enough.

If desired, the analyzer may be used with its plane parallel to the plane of the polarizer, when the background is bright instead of black and extinctions and reinforcements due to interference are interchanged.

The use of white light instead of monochromatic light results in chromatic interference effects. These are due to the different refractive indices of the fiber for the different wavelengths or colors. Thus, when light of one wavelength is extinguished by interference, light of other wavelengths is partly or completely reinforced. With very thin fibers and small double refraction no wavelength completely interferes, so that a grayish white color is seen; but with greater retardations one or another wavelength is extinguished. The effect of this is to subtract this color from the white light. Consequently, whenever a particular color, for example, green, is destructively interfering, the color that is seen is the complement of green or negative green. Destructive interference of green light appears as purple, interference of violet appears as yellow, etc.

As destructive interference can take place at a retardation of one, two, three, or more half wavelengths, the same wavelength will be extinguished by different thicknesses of material. As a consequence, across the varying thicknesses of a fiber the same wavelength will be extinguished several times with

a thick fiber. When using monochromatic light this appears as a series of dark and bright bands, but when using white light a spectrum effect is obtained. Each color is not a true spectrum color, but the remainder of the spectrum minus the color undergoing destructive interference. This "complementary" spectrum may repeat several times between the edges and the center of the thick fiber. Each repetition is called an order. Exactly the same series of colors is not obtained with all substances, since the relative refractive index is not the same for all substances. However, the colors of the first and second orders are sufficiently universal to be used for all substances. The retardation of the complementary colors of the wavelengths extinguished are set out in Table III. As the actual colors seen are always white minus some particular wavelength, they vary with the color of the white light. Thus, the colors seen by daylight are not the same as those seen by artificial light.

TABLE III

TABLE OF POLARIZATION COLORS (15)

Retardation in Micrometers for λ = 589.3 nm.	Interference Colors Between Crossed Nicols	Order
0.000	Black	
0.040	Iron-gray	
0.097	Lavender-gray	
0.158	Grayish-blue	
0.218	Clearer gray	
0.234	Greenish-white	
0.259	White	
0.267	Yellowish-white	I
0.281	Straw-yellow	
0.306	Light yellow	
0.332	Bright yellow	
0.430	Brownish yellow	
0.505	Reddish orange	
0.536	Red	
0.551	Deep red	
0.565	Purple	
0.575	Violet	
0.589	Indigo	
0.664	Blue (sky-blue)	
0.728	Greenish blue	II
0.826	Light green	
0.850	Yellow-green	
0.910	Yellow	
0.948	Orange	
1.101	Violet-red	
1.128	Bluish violet	
1.152	Indigo	
1.258	Greenish blue	III
1.334	Sea-green	
1.426	Greenish yellow	
1.495	Flesh color	
1.534	Carmine	
1.621	Dull purple	
1.652	Violet-gray	
1.682	Grayish blue	
1.771	Dull sea green	IV
1.744	Bluish green	

SIGN OF THE DOUBLE REFRACTION

The simplest way to determine the sign of the double refraction is with a selenite or gypsum plate Red I. Since this has a retardation of 575 nm., the visually brightest light in the greenish yellow is extinguished. Mounted plates of selenite can be obtained with the axis of greatest refractive index marked on the plate. After setting this mark at 45° to the planes of the polarizer and analyzer, a fiber is superimposed above the selenite plate and parallel to the index mark. The Red I of the plate is changed by the retardation. An increase in retardation, showing as one of the colors of the second, or higher orders, indicates that the double refraction of the fiber is positive; but a decrease in the retardation, showing as a lower color of the first order, means that the sign is negative (Figure 37). The color of Red I is very sensitive to slight double refraction, and hence the use of the selenite plate Red I is recommended when searching for traces of double refraction, such as with very thin fibers or fibers with a very small double refraction.

SUBSTAGE DIAPHRAGM

Plane-polarized light becomes elliptically polarized on reflection at any angle other than the normal. This is also true of the refraction of plane-polarized light. Thus, the lenses in the condenser and objectives render plane-polarized light elliptically polarized, and it becomes impossible to obtain perfect extinction with crossed Nicols. In addition, other phenomena which depend on the use of plane-polarized light becomes somewhat confused. With low power lenses the effect is very slight, since then the surfaces of the lenses do not deviate appreciably from the normal. However, the effect of the elliptical polarization produced by the surfaces of high power lenses is very marked.

Elliptical polarization produced by lenses can be avoided if the light incident on their surfaces vibrates parallel or perpendicular to the plane of the incidence of the light on the surfaces. This condition can be attained if a diaphragm with a slit or cross-shaped aperture is used in place of the ordinary substage diaphragm. The diaphragm slit must be arranged parallel or perpendicular to the plane of polarization, and similarly when a cruciform aperture is used. Better results, in terms of resolution, are achieved in this way than by reducing the diaphragm iris in the usual manner. It is important to remember that the numerical aperture, and hence the resolving power and the degree of contrast, will be unlike in different directions. Thus, when a fiber is examined with the slit parallel to the fiber axis the axial striations will be most apparent, and the perpendicular, least apparent.

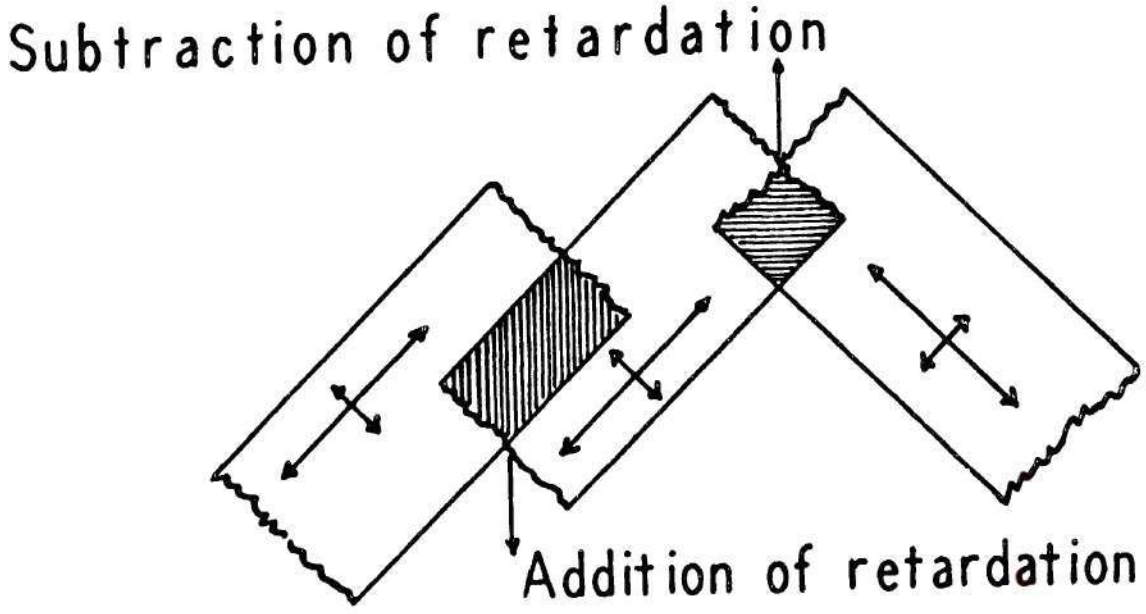

Figure 37. Effect of Superimposition of Double Refractive Material

PLEOCHROISM

The absorption of light in a colored anisotropic substance may vary depending upon the direction of vibration, just as does the refractive index in all anisotropic materials. This phenomenon, observable by means of a Nicol prism, is known as pleochroism. The simplest method for the investigation of pleochroism is to use a polarizer and rotate the sample or the stage.

The pleochroic color changes exhibited by a substance may vary for different views of the specimen. A dichroic substance shows two extremes of hue; a trichroic one, three.

Pleochroism in fibers depends upon the absorption differences of the fibers parallel or perpendicular to the polarization plane. It can be accentuated by staining the fibers with coal tar dyes or with iodine-potassium iodide-metallic salt stains (Table IV). The peculiar characteristic pleochroism of the cotton fiber is illustrated in Table V.

The strong absorption is obtained when the axis of the index ellipse of the fiber is perpendicular to the polarization plane of the Nicol, and the absorption is at a minimum when this axis is parallel with the polarization plane. The contrast between these two positions is very pronounced (black to practically colorless).

Naturally, all dyes do not bring out dichroism. For example, the production of Prussian blue coloring in cellulose does not change the double refraction, and this substance exhibits no dichroism either on glass or in cellulose.

Preston (17) states that the refractive index of the liquid in which the material is mounted makes no difference in the change of the double refraction produced by dyeing. For an equal amount of dyestuff present at any given wavelength, the change of double refraction was independent of the magnitude or sign of the double refraction.

THE POLARIZATION MICROSCOPE (4, 11)

Equipment items which are essential, in varying degree, to the polarization microscope for all around work are:

1. Polarizer and analyzer.

2. Mountings to permit rotation and definite orientation of the polarizing prisms.

3. Cross-haired eyepiece, aligned to indicate orientation of polarizer and analyzer.

4. Rotating graduated stage, with provision for adjusting rotation concentric with the center of the field.

5. Compensators, with provision for inserting them in a slot or otherwise.

6. Revolving nosepiece, with accurately centered, or centerable, openings.

7. Condenser above the polarizer.

8. Bertrand lens.

TABLE IV

THE PLEOCHROISM OF FLAX, HEMP, NETTLE FIBER, RAMIE AND RAYONS EXCEPT ACETATE (12)

Dyeing of Fiber with	Color of Fiber without Nicol	Axis Color[a] After Inserting Nicols		Base Color[b] After Inserting Nicols	
		Without	With	Without	With
		GIBS RED III		GIBS RED III	
Congo red	Red	Dark red	Violet red	Weak red to colorless	Greenish yellow
Benzoazurin	Blue	Dark blue	Red-violet	Light blue to colorless	Blue-green
Diazo brown	Brown	Violet brown	Violet red	Weak yellow-brown	Yellow-green
Benzo brown	Yellow-brown	Reddish brown	Brick red	Light yellow	Greenish-yellow
Methylene blue	Blue, greenish	Greenish blue	Blue, reddish	Light blue	Blue-green
Safranin	Rose	Violet red	Violet red	Violet red, orange tint	Light violet red
Chlorozinc iodide	Dirty violet	Black violet	Violet red	Light red violet to colorless	Green

[a] Fiber perpendicular to the polarization plane of the Nicols.

[b] Fiber parallel to the polarization plane of the Nicols.

TABLE V

PLEOCHROISM OF COTTON AFTER DYEING WITH DIANIL BLUE (12)

		Spiral stripes running from left under to right over (Upper half membrane)	Transition Zone	Spiral stripes running from right under to left over (Upper half membrane)
Without Polarizer			Uniform Light Blue	
Without Gibs Plate	0°	Light blue	White	Light blue
	+45°	Whitish blue	Light blue	Dark blue
	90°	Light blue	Dark blue	Light blue
	-45°	Dark blue	Light blue	Whitish blue
With Polarizer				
With Gibs Plate Red III (+45°)	0°	Blue-green	Blue-green (moderately full)	Blue-green
	+45°	Blue-green	Indistinct Transition	New red
	90°	New red	New red (moderately full)	New red
	-45°	New red	Indistinct Transition	Blue-green
Between crossed Nicols				
	0°	Greenish blue	Black	Greenish blue
	+45°	White	Olive-green	Dark blue
	90°	Greenish blue	Black	Greenish blue
	-45°	Dark blue	Olive-green	White

The greater the number of these features incorporated in the microscope, the greater will be the variety of qualitative and quantitative determinations which can be made with it. However, considerable qualitative work, particularly in fiber microscopy, can be made with an ordinary research-type microscope with a rotating stage, a polarizer and analyzer, and two or three selenite plates; or even with Polaroid accessories such as disk polarizer, cap analyzer, first-order red retardation plate, and quarter-wave retardation plate.

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CHAPTER IV

MICROSCOPE EQUIPMENT REQUIRED IN THE PULP AND PAPER MILL

In preparing specifications for microscopic equipment for general use in pulp, paper, and fiber microscopy laboratories, the following points must be considered:

1. The different uses of the microscope in the laboratory and the relative amount of each type of work.

2. The training and experience of the person in charge of this work and his ability and opportunity to train others to perform routine analyses and tests.

3. The cost of the equipment and the possibility of extending its usefulness by adding accessories, without having to buy separate instruments for each type of work.

USES OF THE MICROSCOPE

The common uses of the microscope in the pulp and paper mill laboratory are: wood identification and study of chip characteristics, fiber identification, degree of uniformity of cooking and bleaching, fiber analysis of paper furnishes, speck and dirt analysis, fiber dimension studies, beating characteristic studies, particle size determination of fillers and pigments, qualitative study of sizing and coating, fluorescence analysis, surface characteristics of paper and board, examination of printing, identification of glues, plastics, and resins, study of microtome sections of fibers, paper and board, photomicrography, textile microscopy, metallography, microbiology, examination of deteriorated wires and felts, and chemical microscopy.

Although the trained microscopist can use the microscope in many other ways, it must be remembered that in many mills the microscope usually is used for only one or two of the above phases, if at all. Further use of the microscope in such a laboratory depends upon the training and experience of the microscopist and upon his and his supervisor's ability to find useful areas for its application.

Therefore, the initial microscope to be recommended for these laboratories must be rugged, reasonably priced, have a general usefulness, and be so constructed that accessories can be added easily as required.

SPECIFICATIONS FOR A GENERAL LABORATORY MICROSCOPE

Rugged arm and pillar, a square horseshoe base to fit solidly on the optical bed of a photomicrographic camera.

Monocular body tube with adjustable draw tube, and interchangeable with binocular tube. (A binocular tube is recommended as additional equipment for laboratories which have a large amount of fiber analysis.)

Coarse and fine adjustment of body tube.

Triple removable nosepiece.

Mechanical stage with millimeter scales for movements in both directions.

Substage arranged so that a polarizer and any type of condenser can be interchanged with the Abbé condenser.

Objectives: 24-mm., 16-mm., 8-mm., 4-mm., and 2-mm. achromatic, as well as one or two Micro Tessar lenses.

Eyepieces: One 10X Huygenian eyepiece equipped with a micrometer scale. The middle division mark should be longer than the others so that the eyepiece can be used both for counting and linear measurements. If a binocular tube is provided, an extra 10X Huygenian eyepiece is necessary. For photomicrography 5X and 10X Hyperplane eyepieces, as well as at least one Ampliplane, Ultraplane or Homal eyepiece should be obtained.

MICROTOMES

A well constructed sliding microtome and a hand microtome are useful. Several microtome knives and sharpening facilities, of course, are also needed.

PHOTOMICROGRAPHIC CAMERA

One of the less expensive models, 5 x 7-inch film, but equipped with an illuminator having an adjustable condenser. In many cases, a smaller film size is satisfactory and a set-up which permits continuous observation of the field to be photographed is to be recommended. A Polaroid Land camera in a suitable attachment has been used successfully.

DAYLIGHT FLUORESCENT LAMP

A lamp, equipped with an 18-inch fluorescent tube, placed 12 to 18 inches from the microscope mirror, gives excellent illumination and color correction, and should be insisted on as standard illumination for fiber analysis.

VARIABLE ANGLE SURFACE ILLUMINATOR

This illuminator is of value for the determination of the relative numerical evaluation of the surface texture of papers and for taking photomicrographs of the same.

ACCESSORIES (to be obtained as needed)

Stereomicroscope, stage micrometer, wide-field microscope lamp, micrometer eyepiece or Filar micrometer (depending upon type of measurements), vertical illuminator, disk polarizer and cap analyzer, first order and quarter wave retardation plates, paraboloid condenser, apochromatic and fluorite objectives, compensating eyepieces, visual, Rheinberg, and Wratten "M" filters.

In addition, the microscopist will require forceps, needles, slides, cover glasses, cells, pipettes, mounting blocks, reagents, stains, and a number of other items, most of which can be found in any regularly equipped laboratory. The following chapters will suggest equipment and accessories which will be needed as the work progresses, and the catalogs of the instrument makers and supply houses will also suggest additional equipment which will facilitate the work.

CHAPTER V

MICROMETRY

INTRODUCTION

Measurements of microscopic objects may be linear, area, or volume determinations, depending upon the character of the material under investigation. However, to appreciate the full meaning of such measurements, one must get in the habit of thinking not only of the data obtainable by the methods to be described in relation to small microscopic or laboratory samples, but of their relationship to the mass the material represents in daily life and the interrelationship such masses may have to each other.

To illustrate, let us consider the micrometric data for two coniferous bleached sulfite pulps. By standard measurement methods it was found that the arithmetic average fiber length for Sample A was 0.49 mm., and the width was 0.024 mm.; for Sample B the arithmetic average fiber length was 0.85 mm.; and the width was 0.028 mm.

From a microscopic point of view these are quite some statistical differences, but probably not of much value to the practical man in the mill. But if we consider the number of fibers per gram of pulp for these samples, the data obtained begin to have some practical meaning. Sample A had 9,100,000 fibers per gram of pulp and Sample B 8,900,000 fibers per gram, which means that the total fiber length in a gram of pulp for Sample A is 4,400 meters and the total projected area 0.100 square meters; and for Sample B the total fiber length per gram is 7,500 meters and the projected area 0.200 square meters.

These data give a little better concept of the micrometric differences between the samples; and the same data, translated to a ton of pulp, show that the total fiber length in a ton of pulp for Sample A is equal to 2,720,000 miles, and the area is 230 acres; for Sample B the total length of the fibers in a ton of pulp is 4,630,000 miles, and the area 500 acres (The fiber surface area is 500 acres and 1000 to 1500 acres, respectively.)

Such data, to the mill men, will have some practical meaning as regards refining, sizing, coloring, etc., of the two pulps; and the data also emphasize the necessity for utmost care in the determination of the micrometric data.

UNIT OF MICROSCOPIC MEASUREMENTS

The unit of microscopic linear measure is the micrometer (μm.) or the nanometer (nm.), although results are frequently expressed as millimeters (mm.). The older term micron (μ) is synonymous with micrometer, and millimicron (mμ) with nanometer.

ACCURACY OF LINEAR MICROSCOPIC MEASUREMENTS

The limit of the precision depends, apart from the mechanical inaccuracy of the apparatus used, upon the sharpness and fineness of the scale of the micrometer, the points of the object between which the measurements are made, the coarseness of the image of the markings on the micrometer scale, and the extent to which they are magnified. The outlines and details of the object vary in apparent width, and depend upon the focus, the illumination, the refractive index of the preparation, and the resolving power of the microscope. The limit is finally dependent upon the breadth of the dark diffraction line which outlines microscopic structures, and is approximately equivalent to the resolving power of the optical system.

METHODS OF LINEAR MEASUREMENTS

Measurement of linear dimensions by means of the microscope depends upon the comparison of the image of the object with a scale of known value and can be done by any of the following methods:

METHOD 1. VISUAL ESTIMATION BASED ON A KNOWLEDGE OF THE MAGNIFYING POWER OF THE MICROSCOPE

The magnification of the microscope is calculated as the ratio of the diameter or length of the image to that of the object. For visual work this image is considered to be 250 mm. from the eye. If the magnification is known, the true size of the object is readily estimated.

METHOD 2. MEASUREMENTS OBTAINED BY MEANS OF A STAGE MICROMETER AND A DRAWING CAMERA (CAMERA LUCIDA)

Camera Lucida is a device which is attached above the eyepiece of the microscope to permit simultaneous observation on the stage and of external objects (Figure 38).

The figure shows diagrammatically the path of the light rays, the dotted lines indicating the image-forming rays from the drawing paper BB reflected by the mirror M, to the reflecting surface of the Abbé prism P, and thence to the eye of the observer. The solid lines indicate the image-forming rays from the preparation upon the stage of the microscope, passing through the aperture (ef) also reaching the eye. Since the observer can see both the vertical image of the preparation and the drawing paper, the outlines and many details of the object may be traced upon the paper with a pencil.

Unless the opening in "ef" is placed at the eyepoint, considerable light will be lost and the field will be restricted. Before attaching the drawing camera always first ascertain the position of the eyepoint.

In order to equalize the light reaching the eye from preparation and drawing paper, a series of dark glasses of graded transmission are mounted so as to turn into position, by a ring between the prism and paper, and a ring between prism and eyepiece.

A Camera Lucida serves not only for drawing but is most useful in micrometry and quantitative analysis; in reading thermometers when melting, boiling or subliming points are determined; or in reading scales of small voltmeters when observations are being made; for upon looking into the microscope, both the preparation and the scale of the instrument may be seen.

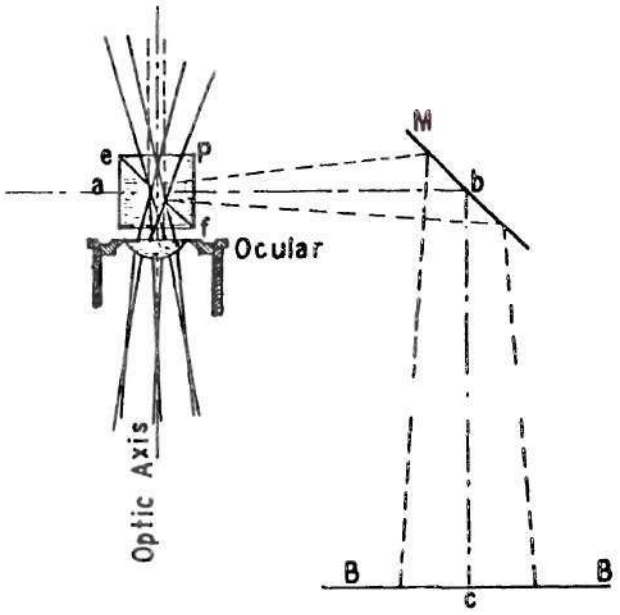

Figure 38. Paths of Rays in Abbé Drawing Camera

Drawings to scale may be prepared according to the above procedure. A stage micrometer is then substituted for the preparation under the microscope, and its divisions are traced on the drawing without altering any of the factors governing magnification. The scale thus drawn indicates directly the various dimensions of the object, and may be used for micrometry.

If other drawings are to be made to the same scale, the factors governing the magnification should be recorded, and the original conditions duplicated each time.

METHOD 3. MEASUREMENTS OBTAINED BY DIRECT COMPARISON OF OBJECTS AND THE GRADUATED MECHANICAL SCALE

A graduated mechanical scale is applicable to measurements of objects larger than the field of the microscope. One end of the specimen is placed beneath the cross hairs of the eyepiece, and the scale of the mechanical

stage is read accurately by means of its vernier. The specimen is moved so as to bring the other end to the same position and the scale reading of the mechanical stage is taken again; the difference gives the length of the specimen. It is necessary that the graduation of the mechanical stage be checked directly against an accurately ruled scale, or against a stage micrometer by the converse of this method. The diameter of the field of view of the microscope may be determined by a procedure similar to this.

METHOD 4. MEASUREMENTS OBTAINED BY MICROMETER EYEPIECES

In order to render object and scale simultaneously visible, a scale may be placed at the lower focal plane of the eyepiece, or specially constructed micrometer eyepieces may be used.

The number of scale divisions covered by the image gives a value for the image only, but not for the object, since the size of the image can be varied without changing the size of the divisions. It is therefore necessary to ascertain the value of the divisions of the micrometer scale with respect to each objective and eyepiece used, for one or more definite tube lengths.

Calibration of Micrometer Eyepiece

Focus the eye lens of the eyepiece so that the graduation of the scale becomes clear and distinct. Lay the stage micrometer upon the stage and move it until the center of the rulings falls in the optical axis of the microscope; focus carefully and adjust the micrometer until a line coincides with a line of the eyepiece scale included between one or more divisions of the stage micrometer. Divide the value of the stage scale by the number just obtained. The quotient gives the value of one eyepiece division.

The determination of the eyepiece micrometer value may be made more exact by eliminating fractions. This is accomplished by altering the ratios between the images of the two scales through a change in the position of the draw tube until the whole number of divisions of the one scale is equivalent to a whole number of the divisions of the other scale.

Since, when high power objectives are used, the rulings of the stage micrometer usually appear as very thick and coarse lines, the eyepiece micrometer scale rulings must be aligned with the center of the coarser rulings of the stage micrometer, or at the right or left of them, but always in the same relative position.

By comparing as many divisions of each scale as possible any errors due to the width of the lines will be made more noticeable and may be corrected more perfectly.

Where delicate measurements are required and filar micrometer eyepieces are used, it is desirable to ensure the best possible resolution and definition; thus, a constant tube length (usually 160 mm.) should be used. This, of course, must be what the instrument manufacturer used. If this is done, it may only infrequently be possible to get a convenient integral number of divisions to coincide. However, this may be sacrificed in favor of accuracy of measurements and sharpness of image.

METHOD 5. PROJECTING A SCALE OF KNOWN VALUE INTO THE FIELD OF VIEW BY MEANS OF THE SUBSTAGE CONDENSER

By the use of a ground glass screen, ruled either with a linear scale or in squares and placed in front of the lamp, an image of the scale can be projected into the field of view by means of the mirror and the substage condenser. The ruled screen can be arranged so that it can be moved backwards or forwards in front of the lamp and clamped in any desired position, thus obviating the need of altering the focus of the condenser once the latter has been located satisfactorily for observation.

The value of the scales are determined for three or more positions of the graduated strip and the results plotted upon coordinate paper. This curve may be employed then for further measurements. The nearer the scale is to the microscope the greater will be the magnitude of the scale image. Once the curve is obtained, we have at our command a device for accurate measurements by means of a scale whose divisions are variable at will between wide limits.

This method is a simple and very convenient one which might be used far more than it is. It possesses one big advantage over the eyepiece micrometer, in that no matter what objectives, eyepieces or tube length are used, direct readings can still be taken, as both object and scale are magnified together. It is essential that the scale not be moved during the readings, as otherwise a recalibration is required.

A contrivance can be designed to take several different scales, or a large scale can be designed on which may be engraved two or more linear scales; and, in addition, a series of radiating lines with the angles between them clearly marked, a series of angles of 20, 30, 45, 60, and 90° being convenient for estimating the angle at which one object or part of an object lies to another.

A blank space should be left in the center of the plate in order to give a clear field when required. If this plate is not too large, and is illuminated evenly and clearly, then, by a simple adjustment of the mirror, any of the desired scales can be brought into the field of view as required. A sketch of such a universal scale (1) is shown in Figure 39.

The area of surface of these scales will vary with the magnification, and it may be necessary to make use of two such screens, one for low power and the other for high power work.

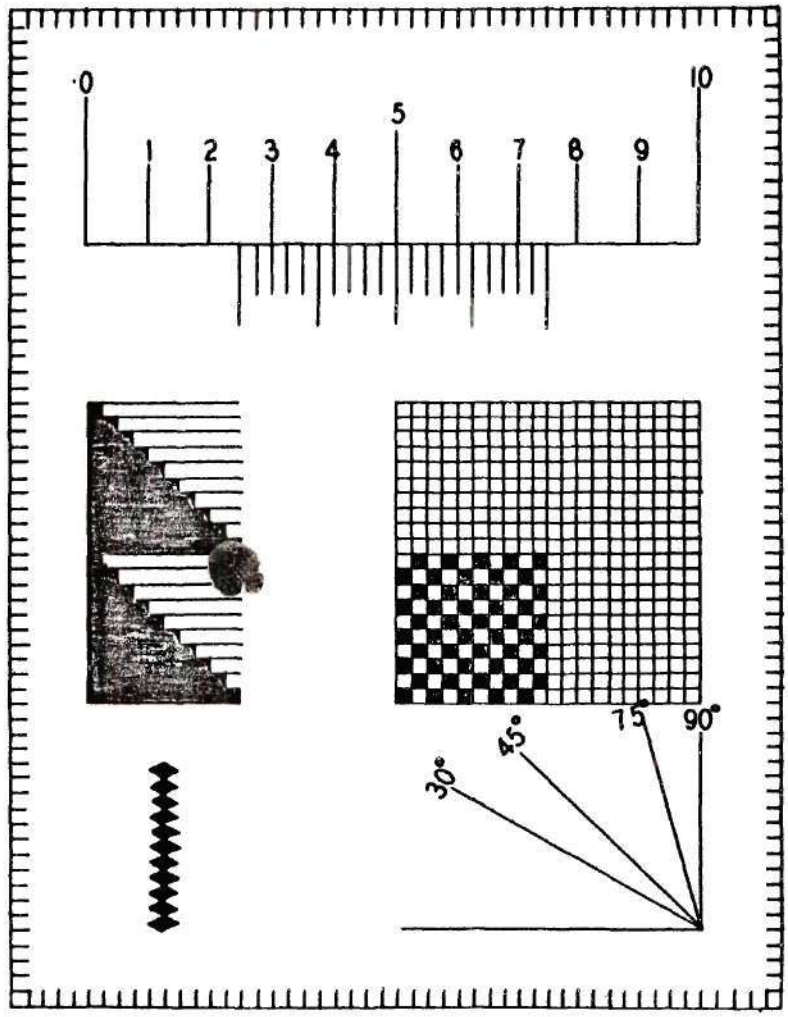

Figure 39. Diagram of Universal Scale

METHOD 6. MEASUREMENT OF PROJECTED REAL IMAGES

If the magnification of a real image projected by the microscope is known, any distance on that image may be measured directly with a scale, and its actual size computed. Measurements on the ground glass of the camera or on a projection screen, or an enlarged photomicrograph are particularly useful in case a great number of measurements have to be taken.

METHOD 7. MEASUREMENTS BY MEANS OF THE GRADUATED FINE ADJUSTMENT

The graduated fine adjustment of the microscope is used for depth or thickness measurements of objects. The value of the divisions of the fine adjustment, as a rule, is marked on the barrel of the adjustment screw. The measurements are made by first focusing on the bottom part of the object, and then on the top, and the distance moved read from the micrometer screw.

This, at first, may seem to be very simple, but there are many factors to be considered, as: (2, 3)

1. The mechanical correctness of the equipment
2. The relative transparency and shape of the object
3. The refractive indices of the object and the mounting medium
4. True axial or annular illumination
5. The focal depth of the objective
6. The depth of accommodation of the observer.

As to Point 1, it is necessary to have mechanically correct measuring equipment. Not all types of construction of the micrometer screws are mechanically correct; some types are better than others. It is possible to make fairly accurate measurements if only the middle part of the micrometer screw movement is used, and it is also of importance that the measurements should be made only by raising of the microscope tube.

A simple method for checking the micrometer screw is as follows: An object slide is marked with an India ink dot or with a diamond scratch and placed on the stage. The objective is placed on the top surface of the slide, and then, with the micrometer screw, carefully raised until the upper part of the line is in sharp focus. The object slide is covered with another object slide or with a cover glass (0.18 mm. thickness) which also has been marked with an ink line or diamond scratch. The tube again is raised with the micrometer screw until the top of the line is in sharp focus. By the thickness and number of the object slides or cover glasses the value of the micrometer screw divisions can easily be determined.

As to Point 2, the measurement of opaque objects – where it is difficult to see when the middle part of the object obstructs the vision of the bottom plane; focusing must be done first on the bottom plane of the object slide. The difference is obtained by focusing on the top plane of the object, raising the objective by means of the micrometer screw. With transparent objects it is necessary to emphasize the lower and upper planes of the object by coloring or relief.

Regarding Point 3, it is very important to know the refractive index of the object, as the measured thickness only checks with the real thickness when

the object has the same refractive index as the space between the object and the objective. If the refractive index of the object is greater than the medium, the measured value will be too small.

If, for example, a piece of a cover glass is used as an object, and this is marked on the upper and lower surface with an India ink mark, the visual thickness, measured with the micrometer screw, will only be CD (see Figure 40) as against the real thickness, CE, because the light rays leaving the object from E will be bent so that they look as though they originated from the higher Point D.

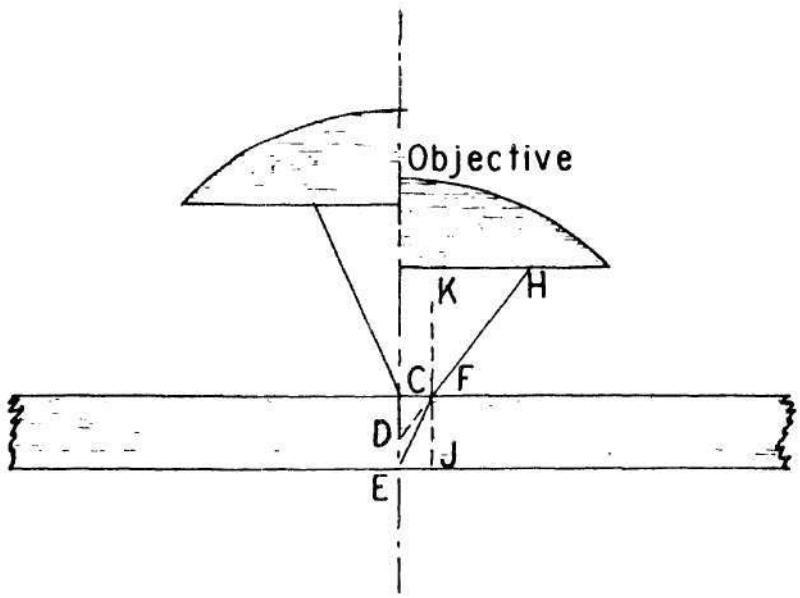

Figure 40. Effect of the Refractive Index on Depth Measurements

The amount of this difference depends naturally upon the refractive index of the object and is easily determined. If the true thickness is "$\underline{d}$" and the visual thickness "δ", then $\underline{d} = \underline{n}\delta$. The true thickness also is determined when the measured thickness is multiplied by the refractive index of the object. Most of the indices are approximately 1.4. This may be done with a probable error of not more than about 5%. This method is to be used when the object is covered with a cover glass. If water or oil immersion is used, then the refractive index of the immersion fluid must be used. The system then is $\underline{d} = \frac{\underline{n}\delta}{\underline{n}'}$, when $\underline{n}$ is the refractive index of the object and

n' the refractive index of the mounting medium. (If the refractive index of the object is lower than that of the mounting medium, the visual value would be too high. The calculation of the true value is done then by the equation just given.) By failing to consider the refractive index, errors of 50% can easily be made.

Regarding Point 4, it is necessary, except in special instances, to consider axial transmitted light.

Regarding Point 5, all object points not sharply focused in the focal plane will look like a number of concentric circles. The size of these circles, as shown in Figure 41, depends upon the distance from the object point to the theoretical focal plane E and also upon the aperture of the light pencil. The focal depth of the optical system can be numerically expressed then when the maximum size of the circles is determined, and also the size of the circles that are so small as to appear as points.

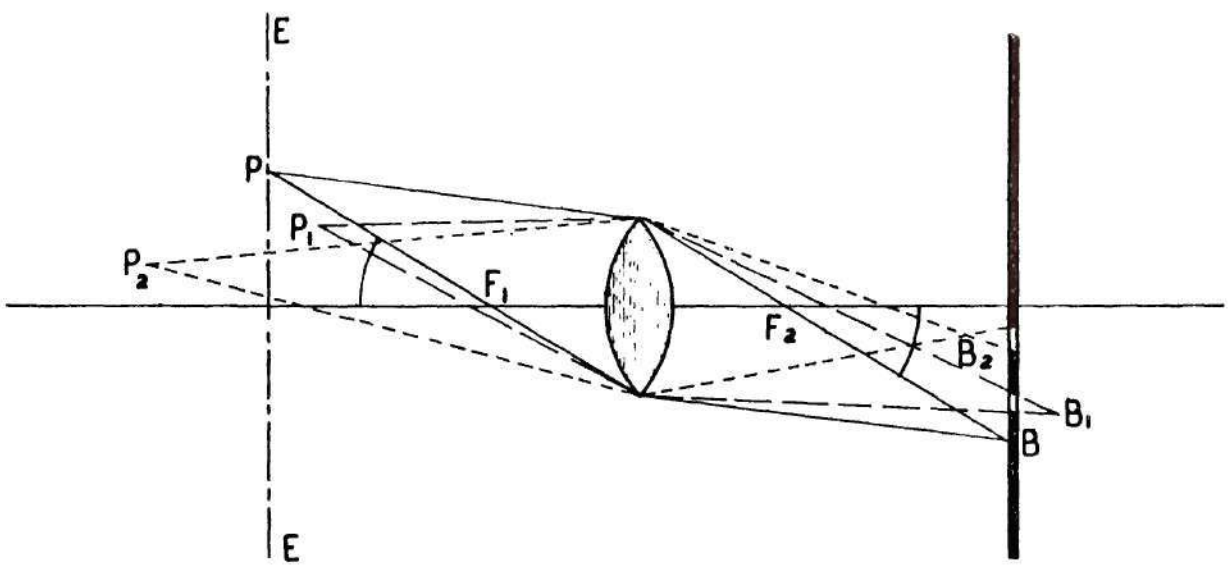

Figure 41. Circle of Confusion Caused by the Focal Planes

The depth of focus or penetrating power of an objective can, as was shown in Chapter I under objectives, be expressed by the following equation:

$$D = \frac{n\ s}{(N.A.)M}$$

where D is the depth of focus, n the index of refraction, s the circle of confusion, and M the magnification; s is generally accepted as being equal to 0.254 mm.

To get an idea of the size of the focal depth, the values in Table VI have been calculated for the normal equipment of most microscopes by fixing the size of the circle of confusion as equal to 0.250 mm.

TABLE VI

FOCAL DEPTH OF NORMAL MICROSCOPIC OPTICS (in μm.)

(The figures in parentheses give the magnification)

Objective	N.A.	Ocular			
		5X	8X	12X	15X
16 mm.	0.25	20.0 (50)	12.0 (82)	8.0 (123)	6.0 (160)
4 mm.	0.65	2.4 (165)	1.5 (265)	0.98 (400)	0.78 (500)
4 mm.	0.85	0.95 (310)	0.59 (500)	0.39 (750)	0.31 (940)
4 mm. water immersion	1.00	1.3 (270)	0.83 (430)	0.56 (650)	0.44 (810)
1.8 mm. oil immersion	1.30	0.78 (450)	0.40 (720)	0.27 (1080)	0.22 (1350)

Table VI also illustrates the advantage of high aperture objectives and high magnification. To make the most of this aperture of the objective, the illumination optics must also be adjusted to fit the optics of the microscope. This means that the condenser must be opened far enough for the identification of the object. If other data are not available the focal depth must be considered undependable.

The error can be reduced considerably if the focusing is done first on the lower and then on the upper plane; then the error of both measurements (upper and lower) will be in the same direction and of the same magnitude, so that the error will not exceed 5% even under the most unfavorable circumstances. It is absolutely wrong to focus on the upper mark by raising and on the lower mark by lowering the objective.

Regarding Point 6, it is a fact that the accommodation plane is an important cause for error and even more so as most microscopists are not able to determine this plane. Just the same it is relatively simple to eliminate this error by using a micrometer eyepiece, as it is only necessary to focus on the points so that the image lies exactly in the plane of the micrometer scale or cross hair. By this method the accommodation will be kept constant.

The depth sharpness of the eye also gives a small variation, as the image may be sharp even if the image plane and division plane differ. This can be overcome by moving the eyes right and left which will show the differences between the two planes.

The summary of this is as follows:

1. Use the largest possible magnification and largest possible N.A. objective.

2. To eliminate the accommodation errors use a micrometer eyepiece and check the focusing by moving the eyes right and left.

3. Focus only by raising the objective to avoid backlash, and select for focusing the points which give even sharpness. If the measurements are repeated start below the first point to find the lower point by raising the objective.

4. All measurements must be repeated several times to reduce the errors.

5. The average of the visual thickness must be converted to the true thickness by the equation $\underline{d} = \underline{n}\delta$ or $\underline{d} = \underline{n}\delta/\underline{n}'$, because this is the largest point of error.

"Mikrokator"

In addition to pointing out sources of error in the previous method, Richards (55) has summarized briefly the precision of thickness measurements made with the microscope. The light-profile technique is suggested for use as a focusing criterion for greater accuracy in combination with a "mikrokator", a mechanical measuring instrument which is attached to the microscope and is capable of 0.1 μm. vertical movement (56).

METHOD 8. VICKERS-A.E.I. IMAGE-SPLITTING MEASURING EYEPIECE

Recently an image-splitting eyepiece has become available for making precise measurements. It consists essentially of a special prism assembly mounted in a conventional compound microscope system. Prisms are linked to a micrometer screw by means of which their angular relation to each other can be varied. When the prism faces are parallel to each other, two images of the object, exactly superimposed and appearing as one, will be visible.

As the micrometer screw is turned, the images move (or shear) across each other. To make an exact measurement, the setting is made so the images are just touching. The micrometer screw is then turned and the images moved across each other until the opposite sides just touch. With calibration, the amount of micrometer screw shear is readily converted into an absolute measurement. Measuring accuracy is as high as 0.125 μm., depending upon the N.A. of the objective. To avoid confusion in a crowded field of view color filters can be introduced, coloring the two images distinctively. The instrument can be used with vertical illumination and phase-contrast (57, 58).

FIBER DIMENSIONS

Under this heading the length/width relationship, the cross-sectional dimensions and the number of fibers in a given weight of material, together with the interrelationship of these data will be carefully considered.

Although the tracheid and fiber dimensions of different trees and plant materials vary, depending upon the geographical location, the climate, and the relative age of the tree (4), the general average dimensions of the tracheids and fibers of different raw materials are of importance to the pulp and paper manufacturer because they will give an idea of the limitations of the finished product and will also enable the selection of the best material for certain specific purposes.

Fortunately, there are published tables giving the approximate range of dimensions of the most important fibrous materials (5, 6, 7).

METHODS OF MAKING LENGTH AND WIDTH DETERMINATIONS

Although the relative length and width of the original tracheids, fibers, seed hairs, bast and vascular bundle fibers are important, the greatest importance of fiber dimensions to us would seem to be in the finished state for use in the different pulp and paper products, and the method used to determine these dimensions and to evaluate the results.

The methods used vary greatly; they include: measuring the bent and twisted fibers by direct microscopic estimation methods, as used by Smith (8) in his investigation of the function of the beater roll in fiber cutting; the different projection methods, using magnifications from 25 to 200 and 300 times, as described by Bergman and Backman (9), Brecht and Mory (10), Schulze (11), Steinschneider, Kross, and Imgrund (12), and Clark (13) and the method of first arranging the fibers parallel to each other on a slide and making accurate microscopic measurements, which was introduced by Graff and Hodgdon in 1918 (14). This method has been further developed and standardized by Graff and others (15, 16, 17, 18).

The above methods are either too inaccurate or too time-consuming, and give little consideration to the relative width of the fibers and the relative percentages of whole and broken fibers, ray cells, and fiber fragments. In any method, although suitable for the purpose at hand, the following facts must be kept in mind if the data obtained are to be valid:

1. The sampling and the preparation of the slides must be carried out in such a manner that the total number of fibers measured represent a true distribution of the different fiber dimensions present.

2. A standard number of measurements should conform with a reasonable time allotment and a minimum of probable error.

3. The method must be reproducible and the personal element so far as possible must be removed.

4. Both the arithmetic and weighted average fiber length, as well as the width, should be considered, and the frequency distribution of the measurements should be expressed in relative weighted percentages as well as in percentages by number.

5. For research purposes, the cross-sectional dimensions as well as the number of fibers per gram of pulp should also be taken into consideration.

In order to retain the accuracy obtained by aligning the fibers parallel to each other on the slide and to simplify and reduce the time necessary for the measurements, a projection apparatus was developed at The Institute of Paper Chemistry which makes it possible not only to determine the length and width of each fiber but also to report if the fibers are whole, broken, ray cells, or fiber fragments (16).

From the tabulated data it is possible, relatively quickly, to determine the arithmetic and weighted average fiber length, the length/width ratios, the

percentage frequency distribution by weight, and the relative weighted amount of whole and broken fibers, ray cells, and fiber fragments.

Preparation of Slides

In earlier works, described by Graff and co-workers, the slides were first prepared by standard methods, and then, before the fibers were straightened out they were stained with a calcium chloride-iodide-iodine stain. This staining method had the following disadvantages: First, the stain faded out before all the fibers were straightened out parallel on the slide with the result that short fibers and fiber fragments were liable to be overlooked. Second, the calcium chloride not only swells the fibers considerably but also has a tendency to make them brittle, thereby not only resulting in exaggerated width measurements, but the fibers were also liable to break during the manipulation.

To overcome these disadvantages the fibers are first stained with the Bright stain*, as described below, before the slides are made. This also has the advantage, that for unbleached fibers, the relative number and total fiber dimensions of raw, medium and well-cooked fibers can be determined.

The stain is made up and used in the following manner: (17)

Solution A: 2.7 g. ferric chloride in 100 ml. distilled water

Solution B: 3.29 g. potassium ferricyanide in 100 ml. distilled water

Solution C: 0.5 g. crude benzopurpurine 4B in 100 ml. of 50% alcohol (dissolved hot).

The paper or pulp is thoroughly disintegrated, and 3.0 g. are squeezed dry between the fingers, placed in 100 ml. of an equal mixture of Solutions A and B at room temperature, and stirred constantly for one minute. The pulp is then strained through a fine crucible or "Varnitized" filter fabric**, squeezed dry between the fingers, and washed once with 500 ml. of distilled water in an Erlenmeyer flask, strained, squeezed dry, put into 100 ml. of Solution C at room temperature, and stirred constantly for two minutes, filtered, squeezed dry, and again washed in 500 ml. of distilled water and diluted until the suspension contains approximately 0.1 g. of pulp in 100 ml. of water, the concentration varying somewhat according to the average fiber length.

When the pulp has been sufficiently diluted, four test tubes, each six inches long and with an outside diameter of 0.75 inch, are filled with this suspension, care being taken to shake the flask thoroughly before filling each tube.

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*We have also found it convenient to stain unbleached pulps with Malachite green and bleached pulps with Congo red.

**Manufactured by the Varniton Company, 5025 W. Washington Blvd., Los Angeles, Calif. 90016

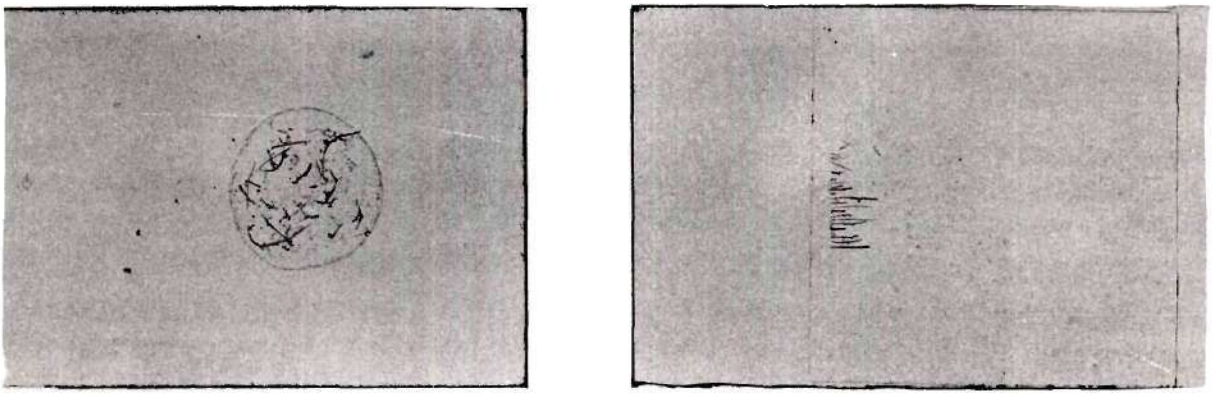
With a dropper having a 5-mm. inside diameter, two drops are taken from each tube, the tube being shaken well before each drop is taken. One drop is placed on the end of a standard slide, using a total of 8 slides. If the suspension has been made properly, each drop should contain about 25 fibers.

The slides are placed carefully into a drying chamber or on a hot plate at about 50°C. and dried thoroughly. If the temperature is too high, the fibers will be cemented too strongly to the slide, and there will be a danger of breaking them during the straightening process.

After drying, the slide is mounted under the dissecting microscope, a drop of a 10% glycerin solution is put on the fibers and left for some time; the slide is then carefully tilted toward one corner, the surplus drop is examined for possible loose fibers, which must be teased back onto the slide, and the surplus solution is removed with filter paper.

The slide is then clamped on the dissecting microscope and the fibers are straightened out one by one with the help of dissecting needles and placed beside each other, parallel to the length of the slide. Care must be taken that every fiber in the original drop is straightened out.

When all the fibers have been straightened, a cover glass is put carefully on the fibers with one edge close to one end of the fibers (Figure 42). With a small pipet, 10% glycerin solution is irrigated under the cover glass from the edge nearest the fiber ends, and the surplus drawn off with a filter paper. The slide is ready now to be placed in the projection arrangement.



A B

Figure 42. Slide for Fiber Length Determination. A, Fibers Before Straightening, B, Finished Slide

Projection Arrangement

The projection arrangement should fulfill the following requirements: The angular aperture of the projection lens must be large enough to cover the whole field of the parallel fibers. As the projection distance is equal to the focal length of a lens times the magnification plus the focal length [FM + F], the lens must have a focal length short enough to prevent undue length of projection at the magnification necessary for accurate determination of the fiber dimensions. The whole equipment should be compact and so arranged that it can be used without disturbing other activities in the room in which it is located.

A convenient arrangement is illustrated in Figure 43. It consists of a projection microscope from which the microscope tube has been removed. In place of an objective, the microscope is equipped with a F 12.5 mm. f 2.8 projection lens. The microscope and illuminant are placed in a boxlike table equipped with a reflecting mirror. The inside of the box is painted a dull black. On the top of the table box and above the reflecting mirror is a ground glass (finely ground) on which a series of lines are drawn at 7.5-mm. intervals, representing the actual space between the lines of 0.1 mm. at 75 diameters magnification. Above the ground glass is a hood (the interior of which is painted dull black), which makes it possible to observe and read the dimensions of the projected fibers without interference from the light in the laboratory.

The slide is put on the stage of the projection microscope and projected on the ground glass under the hood as illustrated in Figure 44, which also shows a transparent micrometer scale (mounted between two lantern slide cover glasses) placed on top of the ground glass (A, Figure 44). This makes possible the determination of the length of each fiber from 0.05 mm. and up and also the estimation or exact measurement of the average width of each fiber to the nearest 0.01 mm.

At a magnification of 75 diameters, ray cells, fiber fragments, broken fibers, and whole fibers can be distinguished and it is possible to enter the length and width of the projected fibers directly as shown in Table VII; in this case, the letters to the left of each length measurement (R, F, B, and W) designate the fibrous material as a ray cell, fiber fragment, broken fiber, or a whole fiber.

A minimum of 200 measurements should be made; if the eight original slides are not sufficient, extra slides must be made. If 200 measurements are reached before a whole slide is finished, the rest of the fibers on that slide are measured and added to the others.

From the data in Table VII, the following can be calculated: the arithmetic and weighted average fiber lengths, the average width, the length/width ratios, and the average area of the fibers, and also the percentage frequency by number and the percentage frequency of the length and the area; these data are given in Table VIII. For all practical purposes, the percentage frequency of the area can be considered as the percentage frequency by weight, assuming that, in one and the same pulp, the relative density is the same for all the fibers.

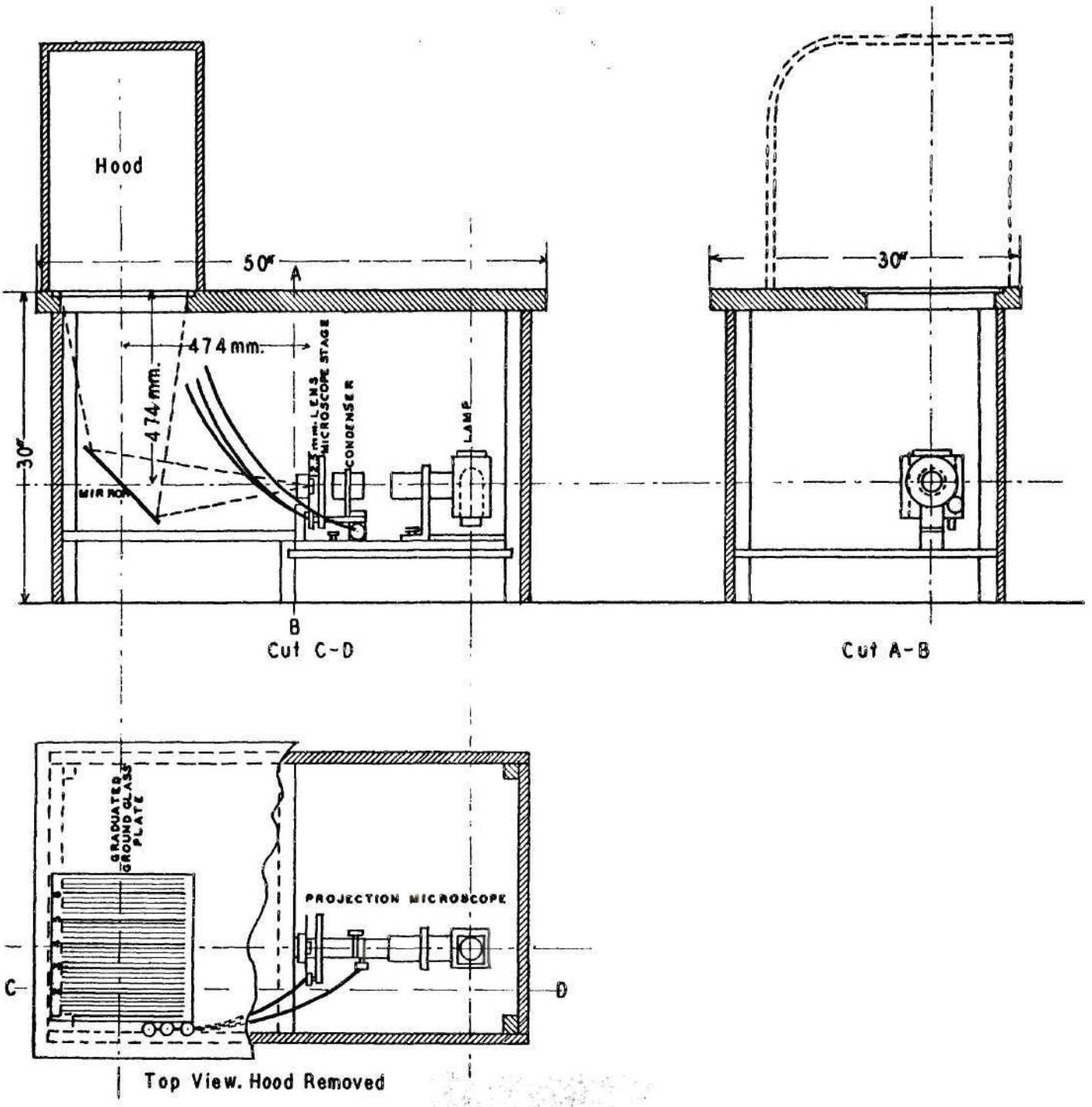


Figure 43. Projection Arrangement for Fiber Length Determination

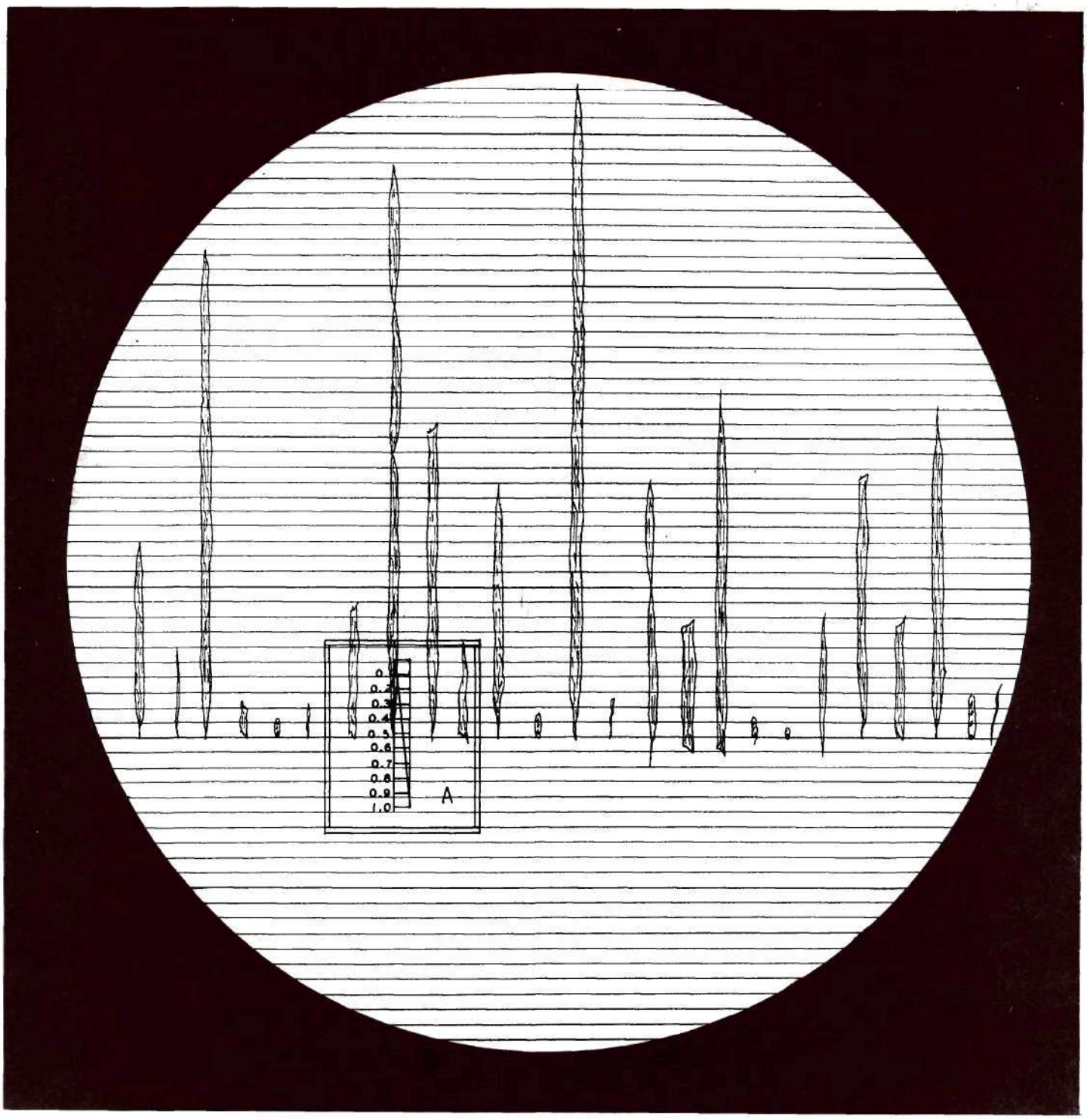


Figure 44. Appearance of Projected Fibers Under the Hood

TABLE VII

OBSERVED READINGS ON SOFTWOOD BLEACHED SULFITE

Length Interval, mm.

0.0-0.1		0.1-0.2		0.2-0.3		0.3-0.4		0.4-0.5		0.5-1.0		1.0-1.5		1.5-2.0		2.0-2.5		2.5-3.0		3.0-3.5		3.5-4.0		4.0-4.5		4.5-5.0	
L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
R0.05	0.02	R0.1	0.01	F0.2	0.01	R0.3	0.02	B0.4	0.04	B0.6	0.05	B0.1	0.04	W1.8	0.04	W2.4	0.04	W2.6	0.03	W3.2	0.05	W3.6	0.06	W4.0	0.06	W4.5	0.05
R0.05	0.01	R0.1	0.01	B0.2	0.05	B0.3	0.02	B0.4	0.03	B0.6	0.04	W1.4	0.04	W1.9	0.04	W2.3	0.05	B2.5	0.05	W3.2	0.04	W3.7	0.04	W4.2	0.05	----------	
R0.05	0.01	R0.1	0.02	F0.2	0.01	B0.3	0.04	----------		B0.8	0.03	B1.2	0.03	B1.9	0.06	W2.2	0.04	W2.8	0.05	W3.1	0.04	----------		----------			
B0.05	0.03	R0.1	0.01	B0.2	0.03	B0.3	0.07	0.8	0.07	B0.5	0.05	B1.0	0.05	W1.7	0.04	W2.2	0.05	W2.5	0.05	B3.1	0.04	7.3	0.10	8.2	0.11		
B0.05	0.02	R0.1	0.01	R0.2	0.02	R0.3	0.03	2/		B0.6	0.02	B1.4	0.04	W1.9	0.06	B2.1	0.06	B2.7	0.06	----------		2/		2/			
B0.05	0.03	F0.1	0.01	R0.2	0.02	R0.3	0.01	0.45	0.035	B0.8	0.05	B1.0	0.04	W1.5	0.02	W2.0	0.04	B2.8	0.05	12.4	0.17	3.65	0.050	4.1	0.055		
R0.05	0.02	R0.1	0.02	F0.2	0.01	----------				B0.5	0.05	W1.0	0.05	B1.6	0.04	W2.3	0.03	W2.6	0.04	4/							
R0.05	0.02	R0.1	0.01	F0.2	0.02	1.8	0.19			B0.7	0.04	W1.0	0.03	----------		W2.3	0.04	----------		3.10	0.043						
R0.05	0.02	R0.1	0.01	R0.2	0.02	6/				W0.6	0.03	B1.3	0.04	12.3	0.30	----------		18.5	0.33								
R0.05	0.02	R0.1	0.01	B0.2	0.03	0.35	0 032			B0.7	0.03	----------		7/		17.8	0.35	7/									
F0.05	0.02	R0.1	0.02	R0.2	0.03					B0.6	0.03	10.4	0.34	1.75	0.043	8/		2.64	0.047								
R0.05	0.02	F0.1	0.02	B0.2	0.03					W0.5	0.03	9/				2.23	0.044										
R0.05	0.02	R0.1	0.01	R0.2	0.02					B0.8	0.04	1.16	0.038														
----------		R0.1	0.02	R0.2	0.03					B0.7	0.03																
0.65	0.26	R0.1	0.01	----------						B0.9	0.06																
13/		F0.1	0.01	2.8	0.33					W0.8	0.04																
0.05	0.020	R0.1	0.01	14/						B0.7	0.04																
		R0.1	0.02	0.25	0.024					B0.7	0.04																
		R0.1	0.02							B0.6	0.03																
		R0.1	0.02	R Ray cell						---	-------																
		F0.1	0.02	F Fiber fragment						1.27	0.73																
		R0.1	0.02	B Broken fiber						19/																	
		R0.1	0.01	W Whole Fiber						0.67	0.038																
		R0.1	0.01																								
		R0.1	0.02																								
		F0.1	0.02																								
		R0.1	0.02																								

		2.7	0.40																								
		27/																									
		0.15	0.015																								

Note: In the columns of this table the fiber length is reported only to the first significant figure, although the second decimal is considered in determining the average value. The number of observations and the average of the length interval have been placed beneath the totals in each column, e.g., in column 1, 13/ is the number of observations and 0.05 and 0.020 are the average.

TABLE VIII

CALCULATED VALUES DERIVED FROM TABLE VII

mm.	Frequency	Percentage by Number, (a)	Average Length, (b) mm.	ab	Percentage of Length	Av. Width, (c) mm.	ac	abc	Percentage of Area	ab^2
0.0-0.1	13	10.75	0.05	0.54	0.57	0.020	0.215	0.0108	0.27	0.027
0.1-0.2	27	22.30	0.15	3.34	3.51	0.015	0.334	0.0501	1.25	0.503
0.2-0.3	14	11.60	0.25	2.90	3.02	0.024	0.278	0.0695	1.73	0.725
0.3-0.4	6	4.96	0.35	1.73	1.82	0.032	0.159	0.0556	1.38	0.605
0.4-0.5	2	1.65	0.45	0.75	0.79	0.035	0.058	0.0261	0.65	0.334
0.5-1.0	19	15.70	0.67	10.50	11.00	0.038	0.595	0.3980	9.90	7.050
1.0-1.5	9	7.45	1.16	8.62	9.05	0.038	0.283	0.3280	8.18	10.100
1.5-2.0	7	5.80	1.75	10.16	10.67	0.043	0.249	0.4350	10.82	17.750
2.0-2.5	8	6.60	2.23	14.70	15.45	0.044	0.291	0.6500	16.20	32.800
2.5-3.0	7	5.80	2.64	15.30	16.10	0.047	0.272	0.7175	17.85	40.500
3.0-3.5	4	3.30	3.10	10.21	10.72	0.043	0.142	0.4400	10.95	31.800
3.5-4.0	2	1.65	3.65	6.02	6.32	0.050	0.083	0.3020	7.50	22.000
4.0-4.5	2	1.65	4.10	6.75	7.10	0.055	0.091	0.3730	9.28	27.750
4.5-5.0	1	0.84	4.50	3.82	4.00	0.050	0.043	0.1635	4.07	17.200
	121			95.33			3.093	4.0191		209.144

Arithmetic average fiber length (a) ..... 0.95 mm.

Weighted average fiber length (b) = $\Sigma ab^2/\Sigma ab$ 2.20 mm.

Average width ..... 0.031 mm.

Length (a)/width ratio 30.6

Length (b)/width ratio 71.0

Average area ..... 0.040 $mm.^2$

The relative amount and frequency distribution by weight of ray cells, fiber fragments, broken fibers, and whole fibers in a pulp can be calculated from the data in Table VII, and plotted as shown in Figure 45.

For the purpose of determining the relative effect of the diameter of the opening of the dropper and the number of measurements to attain a reasonably low percentage probable error for the different calculated factors, seven sets of 100 measurements each were made of softwood unbleached sulfite, using a dropper with a 4.4-mm. opening; 22 sets of 100 measurements each were made of the same pulp, using a dropper with a 5.9-mm. opening; and ten sets of 100 measurements each were made of softwood bleached sulfite, using a 4.4-mm. and a 5.9-mm. dropper, respectively.

Table IX shows the individual results of 17 sets of measurements for softwood unbleached sulfite using a 4.4-mm. dropper, together with the average of each factor for the total number of measurements and the standard deviation (S.D.) and the probable error (P.E.) for each individual set.

Table X shows the average for each factor for the four different experiments, together with the standard deviation and probable error for a total of 100 measurements in each case, and Table XI shows average percentage probable errors for 100, 200, 400, 600, 800, and 1000 measurements. These results are plotted in Figure 46.

The data in Table X indicate that the arithmetic average fiber length for the unbleached pulp was 0.62 mm. using a 4.4-mm. dropper and 0.70 mm. using a dropper with a 5.9-mm. opening; the results for the bleached sulfite show a length of 0.76 mm. for the 4.4-mm. dropper and 0.66 mm. for the 5.9-mm. dropper. As the calculated probable error in all four instances was 0.08 mm., it must be assumed that any dropper opening between 4.5 and 6.0 mm. will give comparative results. The curves in Figure 46 illustrate two significant facts: (1) At least 200 measurements must be made to obtain results with a reasonably low percentage of probable error, as little will be gained going any further, and (2) the percentage of probable error for the weighted average fiber length and for the average fiber width are considerably less than the percentage of probable error for the arithmetic average fiber length. This is important, because the weighted average fiber length and the percentage frequency distribution by weight are, probably, the significant values in fiber length data and because, as shown in previous work (18), the weighted average fiber length is inversely proportional to the number of fibers in a gram of pulp.

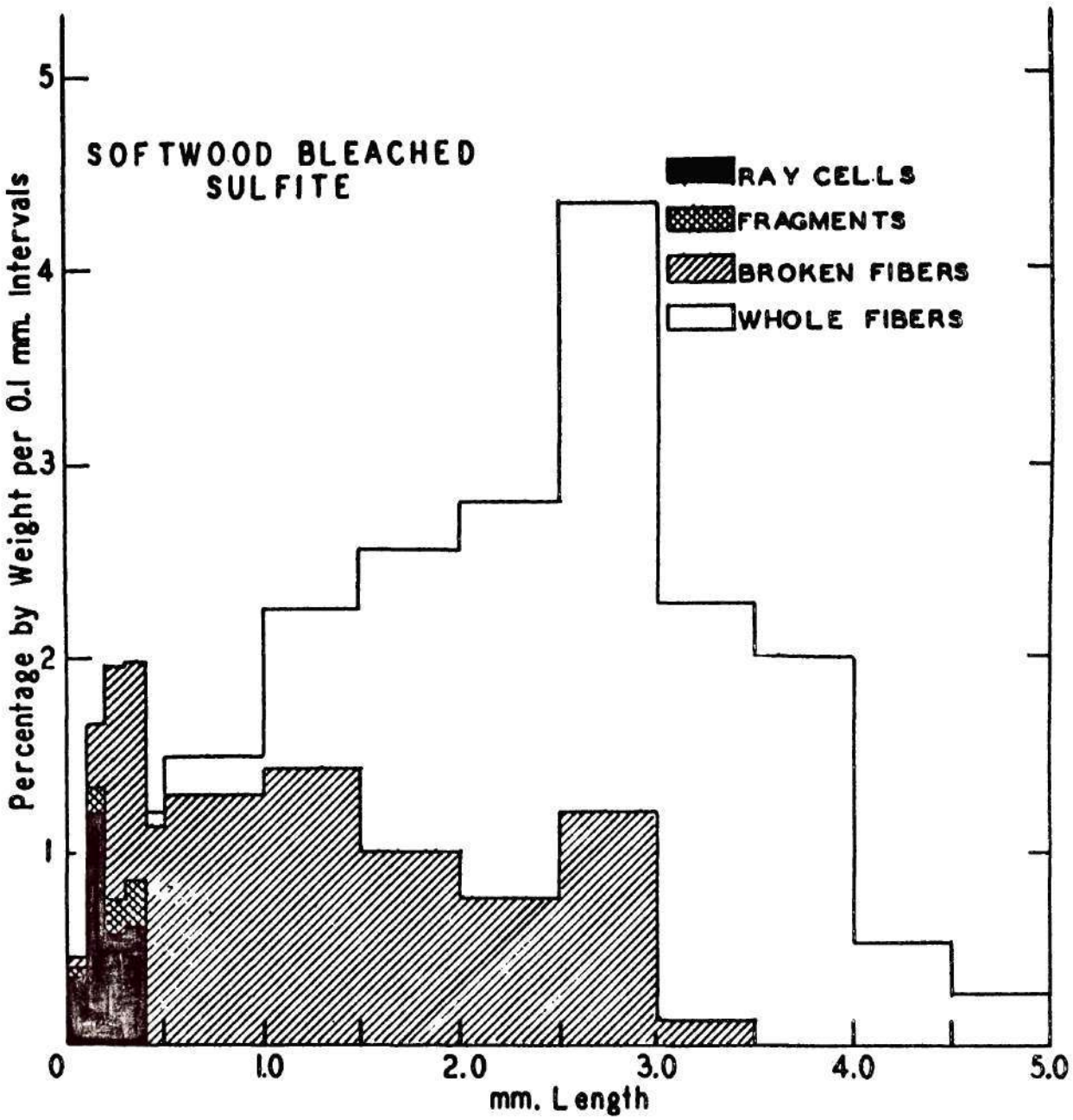


Figure 45. Frequency Distribution by Weight of Ray Cells, Fragments, Broken and Whole Fibers

TABLE IX

LENGTH AND WIDTH BY PROJECTION OF SOFTWOOD UNBLEACHED SULFITE

Dropper 4.4-mm. opening

Set	Arithmetic Av. Fiber Length (a), mm.	Weighted Av. Fiber Length (b), mm.	Average Width, mm.	Length (a)/ Width Ratio	Length (b)/ Width Ratio	Average Area, $mm.^2$
1	0.61	2.12	0.024	25.40	88.50	0.024
2	0.82	2.38	0.029	28.20	82.00	0.034
3	0.54	1.81	0.024	22.50	75.50	0.020
4	0.48	1.32	0.021	22.80	62.85	0.018
5	0.60	2.18	0.025	24.00	87.50	0.024
6	0.57	1.98	0.023	24.80	86.00	0.022
7	0.55	1.94	0.024	22.90	81.00	0.022
8	0.57	2.01	0.021	27.15	95.80	0.020
9	0.57	1.73	0.022	25.80	78.75	0.022
10	0.48	1.94	0.020	24.00	97.00	0.017
11	0.88	2.41	0.030	29.30	80.50	0.039
12	0.48	1.48	0.025	19.20	59.20	0.018
13	0.78	2.03	0.033	23.65	61.50	0.035
14	0.48	1.22	0.026	18.45	47.00	0.028
15	0.74	2.25	0.027	27.40	83.50	0.033
16	0.62	2.04	0.023	27.00	75.60	0.023
17	0.67	1.91	0.024	27.90	59.50	0.023
	10.44	32.75	0.421	420.45	1321.70	0.422
Average	0.62	1.93	0.025	24.80	77.30	0.025
S.D.	0.12	0.33	0.0033	2.94	12.40	0.0064
P.E.	0.08	0.23	0.0023	2.05	8.62	0.0045
S.D., %	19.00	17.00	13.00	12.00	16.00	26.00
P.E., %	13.00	12.00	9.00	8.00	11.00	18.00

TABLE X

RESULTS OF LENGTH AND WIDTH MEASUREMENTS BY PROJECTION

	Arithmetic Av. Fiber Length (a), mm.	Weighted Av. Fiber Length (b), mm.	Average Width, mm.	Length (a)/ Width Ratio	Length (b)/ Width Ratio	Average Area, $mm.^2$
Softwood unbleached sulfite. Dropper 4.4-mm. opening. 17 Sets of 100 measurements						
Averages	0.62	1.93	0.025	24.80	77.30	0.025
S.D.	0.12	0.33	0.0033	2.94	12.40	0.0064
P.E.	0.08	0.23	0.0023	2.05	8.62	0.0045
S.D., %	19.00	17.00	13.00	12.00	16.00	26.00
P.E., %	13.00	12.00	9.00	8.00	11.00	18.00
Softwood unbleached sulfite. Dropper 5.9-mm. opening. 22 Sets of 100 measurements						
Averages	0.70	2.00	0.027	26.00	74.00	0.028
S.D.	0.12	0.28	0.003	4.27	15.80	0.006
P.E.	0.08	0.19	0.002	2.96	11.20	0.004
S.D., %	17.00	14.00	11.00	16.00	21.00	21.00
P.E., %	11.00	10.00	7.00	11.00	15.00	14.00
Softwood bleached sulfite. Dropper 4.4-mm. opening. 10 Sets of 100 measurements						
Averages	0.76	2.12	0.026	29.20	81.60	0.029
S.D.	0.11	0.15	0.0024	2.26	7.33	0.0051
P.E.	0.08	0.11	0.0017	1.53	5.22	0.0036
S.D., %	14.00	7.00	9.00	8.00	9.00	18.00
P.E., %	11.00	5.00	7.00	5.00	6.00	12.00
Softwood bleached sulfite. Dropper 5.9-mm. opening. 10 Sets of 100 measurements						
Averages	0.66	1.98	0.023	28.70	86.00	0.023
S.D.	0.12	0.16	0.0022	3.74	7.90	0.0043
P.E.	0.08	0.11	0.0015	2.65	5.60	0.0031
S.D., %	18.00	8.00	10.00	13.00	9.00	19.00
P.E., %	12.00	6.00	7.00	9.00	7.00	13.00

TABLE XI

AVERAGE PERCENTAGE OF PROBABLE ERRORS FROM 100 TO 1000 MEASUREMENTS

Number of Measurements	Arithmetic Av. Fiber Length (a), %	Weighted Av. Fiber Length (b), %	Average Width, %	Length (a)/ Width Ratio, %	Length (b)/ Width Ratio, %	Average Area, %
100	11.80	8.20	7.50	8.20	9.80	14.20
200	8.20	5.50	5.10	5.80	6.60	9.90
400	5.80	3.90	3.60	4.10	4.70	7.00
600	4.80	3.20	2.90	3.40	3.80	5.70
800	4.10	2.80	2.60	2.90	3.30	5.00
1000	3.70	2.50	2.30	2.60	3.00	4.50

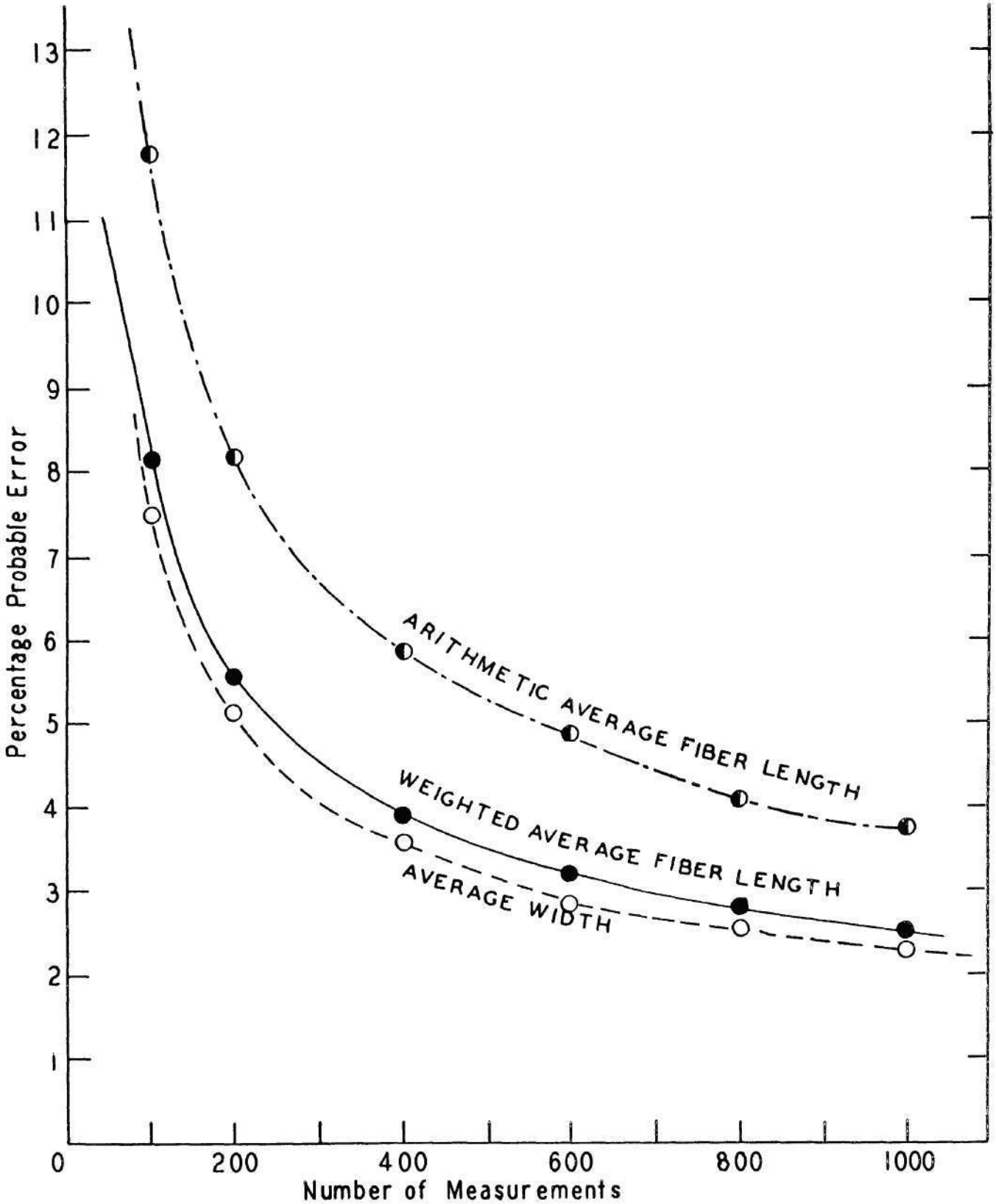


Figure 46. Percentage Probable Error for 100 to 1000 Measurements

From the data presented, we note the following factors:

(1) The relative importance of the arithmetic average and weighted average fiber lengths as well as the length/width ratios of these two expressions has not been investigated; but as the length/width ratio is important both as regards felting and the relative compactness of the finished sheet (19) and, as has already been mentioned, the weighted average fiber length is inversely proportional to the number of fibers in a given weight of pulp, the weighted average length probably is the more important.

(2) A large number of the published data on fiber lengths, in particular the tables giving fiber length data of different woods, bast and vascular bundle fibers, unfortunately give only the minimum, average and maximum length of the fibers with no information on frequency distribution or standard deviation.

Klemm (19) and Schulze (11) suggested the advisability of reporting fiber length dimensions as the percentage by number of short, medium, and long fibers; and Bixler (20) and Graff and Miller (21) demonstrated that fiber length dimensions should be reported as the percentage by number and by weight of short, medium, and long fibers.

From tabulations like that shown in Table VIII, it is relatively simple to determine the percentages of short, medium, and long fibers by number or by weight. Determination by weight should eliminate, to a certain extent, the need for screen fractionation, because the percentages of the relatively short, the very long, and the intermediate lengths can be determined with the assurance of no overlapping of the divisions. However, one must decide the limits of these divisions; in most cases, they will depend upon the pulps to be compared or the purpose of the investigation.

In Table XII are included data on certain pulps studied in earlier work (18) – the arithmetic and weighted average fiber lengths together with the percentages by number and by weight of fibers shorter than 0.5 mm., 0.5 to 1.5-mm. long, and longer than 1.5 mm. Plots of these percentages on triangular charts (Figures 47 and 48) give a clear comparison of the percentages for the selected divisions of short, medium, and long fibers.

Not too many decades ago the beaterman gleaned knowledge of the fiber length of the pulp by passing a stick or rod through it and observing how much fiber was picked up. In 1946, de Montigny and Zborowski (22) devised a grid technique by which a dilute aqueous suspension of fibers is made to flow past a set of fixed vertical blades under standardized conditions. The weight of fibers retained provides an index of fiber length. A definite correlation seems to exist between fiber length distribution and weight retained by the grid for specific pulps (22, 23).

TABLE XII

FIBER DIMENSIONS OF PULPS

No.	Pulps	Arithmetic Length, mm.	Weighted Length, mm.	Percentages by Number from 0.0-0.5 mm., %	Percentages by Number from 0.5-1.5 mm., %	Percentages by Number from 1.5-up mm., %	Percentages by Weight from 0.0-0.5 mm., %	Percentages by Weight from 0.5-1.5 mm., %	Percentages by Weight from 1.5-up mm., %
1	Southern pine unbleached kraft	1.39	3.27	47.00	19.00	34.00	3.46	10.74	85.80
2	Eastern jack pine unbleached kraft	0.63	2.18	68.50	14.00	17.50	6.57	20.38	73.05
3	Western hemlock unbleached kraft	0.80	2.28	67.00	11.20	21.80	9.53	13.11	77.36
4	Western softwood bleached sulfite	0.49	1.96	78.60	11.40	10.00	13.54	23.18	63.28
5	Softwood bleached Mitscherlich sulfite	0.51	2.38	80.50	6.00	13.50	10.07	9.40	80.53
6	Balsam fir unbleached sulfite	0.64	1.65	65.30	17.80	16.90	8.87	25.45	65.68
7	Eastern softwood unbleached sulfite	0.58	1.60	68.50	19.00	12.50	14.47	27.56	57.97
8	Eastern softwood bleached sulfite	0.85	2.30	58.00	22.00	20.00	6.96	26.51	66.57
9	Softwood paper-grade alpha	0.98	2.29	52.70	20.10	27.20	4.81	18.71	76.48
10	Rag filter paper	0.58	0.99	60.00	33.00	7.00	28.78	48.45	22.77
11	Hardwood bleached sulfite	0.18	0.65	90.00	9.00	1.00	46.73	38.54	14.73
12	Hardwood bleached soda	0.33	0.69	72.00	28.00	0.00	35.41	64.59	0.00
13	Hardwood paper-grade alpha	0.35	0.76	69.50	29.50	1.00	23.09	69.70	7.21

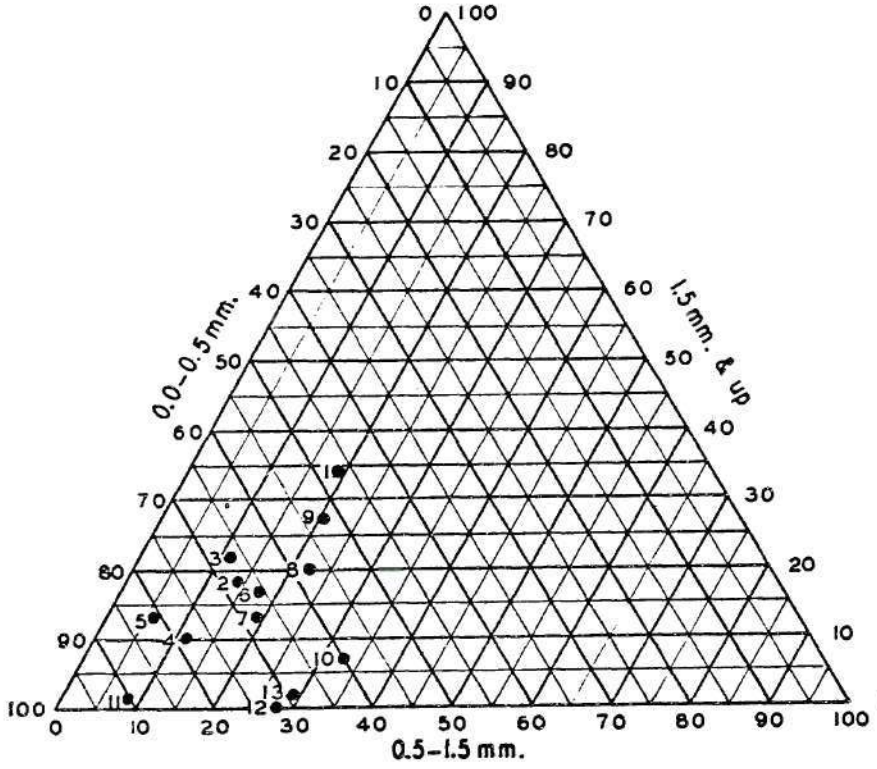


Figure 47. Percentage Distribution by Number of Short, Medium, and Long Fibers

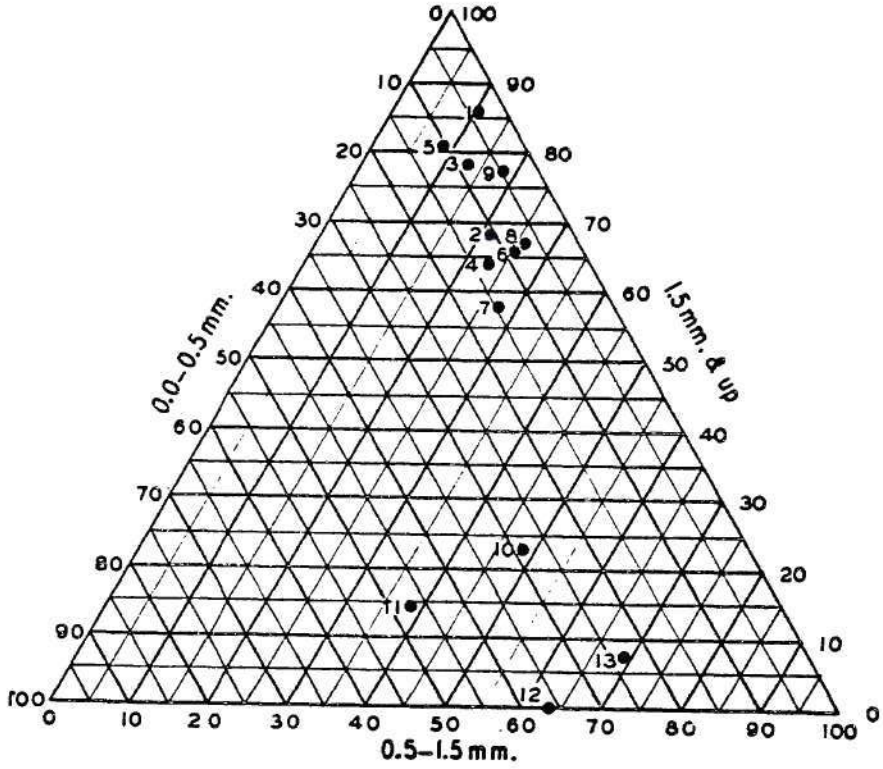


Figure 48. Percentage Distribution by Weight of Short, Medium, and Long Fibers

Screen analysis, fractionation, or classification has been used to give an indication of fiber length in a pulp either alone or in combination with fiber length measurements within a fraction (13, 24, 25, 26, 27). Marpon (28) proposed determination of "length number" obtained by separating the pulp into a long fiber fraction and a short fiber fraction by means of an apparatus resembling a Schopper-Riegler freeness tester.

Attempts have been made in recent years to determine an average fiber length rapidly by determining the number of fibers inside a selected circle and the number of fibers bisected by the circle (29, 30, 59). A derived relationship gives the average fiber length. Others have counted the number of times the projected fibers cross a grid pattern (26, 31, 59).

In addition to the projection methods mentioned previously those of T 232 su-64 (32), Fyfe (33), and Wilson (34) should be referred to. Wilson (34) has given a good summary of methods for fiber length determination in the same article in which he describes his graduated bull's-eye target method.

MEASUREMENT OF FIBER LENGTH WITH A SEMIAUTOMATIC RECORDER

In her review of methods of fiber-length determination, Ilvessalo (52) mentioned a semiautomatic method developed at the Finnish Pulp and Paper Research Institute. A description of the method and the apparatus* has been published (53).

A stained fiber slide is projected from below upon a screen, using 50X magnification. The lengths of the fibers are measured from the projected image by tracing from one end of the fiber to the other with the rotating wheel of a curvimeter. At the end of the fiber, the push lever of the curvimeter is depressed and the curvimeter raised a little. This movement imparts an impulse which records the fiber in the proper length group.

The recorder does the following during the process of measurement: (1) It divides the measured fibers into length groups, and tabulates the number of fibers in each group. The span of a group is 0.2 mm. with the exception of the first two groups, which are 0.1 mm. Although the 1957 model was constructed for a maximum fiber length of 5.4 mm., the machine can be built so that longer fibers may be measured. (2) It gives the total number of the measured fibers. (3) It gives the total length of the measured fibers as a sum of the products obtained by multiplication of the average of every length group by the number of fibers in the group. However, if desired, the first two groups, 0-0.1 and 0.1-0.2, may be recorded by the summing calculator as belonging to one group only, the average of which is 0.1 mm.

The projector has a rotating object stage. To facilitate measuring the field of view, a circular glass plate 2 cm. in diameter and divided into 12 sectors and an inner circle, is attached in the center of the object stage, 0.2 mm. below the slide.

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*Manufactured and distributed by AB Scienta, Kvillegatan 9B, Goteborg, Sweden.

On the sloping measuring table there is a glass plate of 72 cm. x 47 cm., which is covered with a translucent plastic drawing foil to prevent slippage of the curvimeter wheel.

In principle, the fiber slide is prepared according to a method developed by Bergman and Backman (9). All fibers in a field of 2 cm. diameter are measured with the curvimeter, including any crossing the outer circle when more than half of their lengths lie within the line. A fiber field containing 600-850 fibers of an unbleached spruce sulfite pulp is easily measured by a trained operator in 30-40 minutes.

The recorder automatically gives the number of fibers in every length group, and the total number and total length of the measured fibers. These data, plus average of length interval in mm., percentage of fibers by number, and percentage of length per interval, are tabulated and presented in routine work by a distribution curve according to length and by the arithmetic average fiber length, if desirable.

Investigators at the Institute of Paper Manufacture in Darmstadt also have described (54) a semiautomatic fiber length measuring instrument, on which construction was begun early in 1956, as being characterized by the following points: 1. Free mutually independent adjustability for the span of the length intervals. 2. Great accuracy in reading on the recording apparatus the length of each fiber traced. 3. Handiness and lightness of the tracing pencil carrying the curvimeter. 4. Limitation of the fiber tracing speed only by the care necessary for accurate tracing.

Unger and Unger (60) have described a semiautomatic recording device, using a measuring wheel, which classifies the fiber length measurements from 0.1-5.0 mm. into 12 intervals of increasingly greater span with increasing fiber length, rather than the usual equal classes. The frequency curve and length distribution curve may be diagrammed on an oscilloscope and photographed, if desired.

Coulter Counter

A particle content and size distribution measuring instrument – the Coulter Counter – has become commercially available (61). This unique method determines the number and size of particles suspended in an electrically conductive liquid. The suspension flows through a small aperture having an immersed electrode on either side, with particle concentration such that the particles traverse the aperture substantially one at a time. It is claimed to be effective in the range of 0.5 to 500 μm. particle size.

Valley and Morse (62) have devised a modification of a Coulter particle counter for use in determining fiber length distributions rapidly. The modifications include a revised valving arrangement and modified sample holder. Electronically, the Coulter was changed from a volume-sensing to a length-of-time-sensing device. It is possible to run all fibers over a size range of 0.05 to 7 mm. An average fiber length value can be determined to within ± 0.1 mm.

With this modification the instrument can count fibers at only one threshold setting at a time. Preparation and counting of a sample at 10-12 thresholds requires about 1/2 hr. Computations for length-weighted data take additional time but, of course, are highly recommended. Further modification of the electronic circuitry could greatly reduce the counting time.

CROSS-SECTIONAL DIMENSIONS OF FIBERS

The textile microscopists have long appreciated the importance of the different data obtainable by the cross-sectional dimensions of rayon fibers, and use the following definitions and equations in the micrometry of rayons.

Width: The diameter in micrometers of the circle circumscribed about the cross section (W in Figure 49-1).

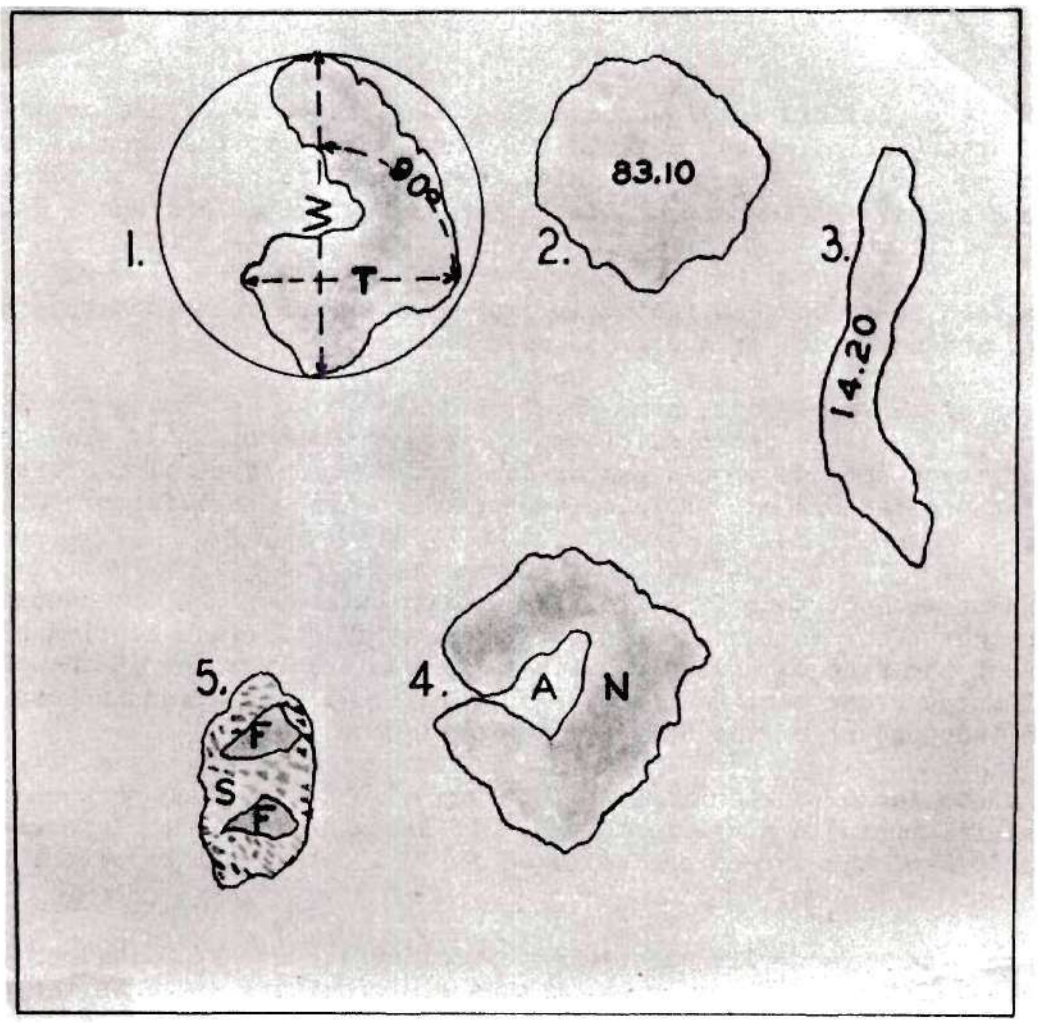

Figure 49. Cross-Sectional Nomenclatures

Thickness: The greatest thickness in micrometers on any perpendicular to the longest line which can be drawn across the cross section (T in Figure 49-1).

Ratio of Width to Thickness: This figure is larger, the more the cross section departs from the circle.

Density: Real and wild silk, 1.37; nitrocellulose, cuprammonium, and viscose, 1.52; acetate, 1.26.

Roundness Factor: The oldest method of roundness factor determination is to inscribe the cross section in a rectangle with its sides parallel to

the greatest width and thickness of the section and calculate the factor as follows: Vr (in percent) = $(F \times 100)/R$, where Vr is the roundness factor in percent, F is the cross-sectional area of the fibers, and R is the area of the rectangle.

Herzog (35) defined the roundness factor as the ratio of the cross-sectional area (including air pockets) of the filament to the area of the circumscribed circle. This may be multiplied by 100 to convert to percent. Thus, for viscose, Vr (in percent) is 10,000 x legal titer/W^2, where W is the width of the filament.

Sometimes the roundness factor is expressed as the relationship between the length and the width of the cross section.

No one of these methods, however, is sufficient to determine the physical characteristics and the cross-sectional formation of rayon (36). The cross section of rayon depends upon a number of chemical and physical factors; a number of these factors can be calculated from the roundness factors of the filaments.

The best method, therefore, for the determination of the roundness factor is to consider the relationship between the area of the cross section of the filament and the area of a circle having a circumference equal to the perimeter of the filament cross section itself. This ratio will be dimensionless, and therefore independent of the units of measurement chosen.

Roundness factors determined in this way do not vary much between the individual filaments in a single thread. It is, therefore, not important which cross sections of the thread are selected for the determination of the roundness factor.

The roundness factor is calculated as follows: $Vr = F/(U^2/4\pi)$ or $(4\pi F)/U^2$, where F is the cross-sectional area in square micrometers and U is the perimeter in micrometers. The area of a circle with the periphery of U is $U^2/4\pi$; if r is the radius of the circle, $U = 2\pi r$ or $r = U/2\pi$, and the area of the circle is $\pi r^2 = U^2/4\pi$. To obtain Vr in percentage, the above value is multiplied by 100.

Gross Area: The area in square micrometers of the cross section of the filament including all air pockets which may be enclosed. In Figure 49-4 it is $N + A$.

Net Area: The gross area minus the area of the enclosed air pockets. In Figure 49-4, it is N.

Legal Titer in Deniers: The weight in grams of 9000 meters of a single filament.

Metric Number: The number of meters of a single filament which will weigh one gram. If F_1 is the net cross-sectional area in square micrometers and S is the density in grams, the metric number equals $1{,}000{,}000/F_1 S$.

Actual Number of Filaments: This number is found by check count.

Given Number of Filaments: The number of holes in the spinneret used.

Commercial Denier: The weight in grams of 9000 meters of the whole thread, regardless of the number of filaments. It is equal to the legal titer times the actual number of filaments in the thread.

Percentage Variation of Any Measurement: One-half of the difference between the means of measurements higher and lower than the general mean. If M is the general mean, M_1 the higher mean (the mean of the data between the general mean and the highest datum), and M_2 the lower mean (the mean of the data between the general mean and the lowest datum), the percentage variation equals $1/2\ [(M_1 - M) + (M - M_2)] \times 100$.

The cross-sectional calculations also enable us to determine two other very important rayon factors: the "covering power" and the "softness factor" of a rayon thread.

The covering power of a thread is the diameter of filaments twisted together on a spindle.

The softness factor is proportional to the relative amount of air in and around the filaments of the thread and is inversely proportional to the legal titer of the filament.

The specific covering power of a thread is the calculated diameter of a thread with a total weight of one denier.

By calculating these factors for a large number of rayon threads with different filament roundness factors and cross-sectional areas, it has been found that there is a marked negative correlation between the average roundness factors of the filaments and the specific covering power of the thread, and a strong positive correlation between the specific covering power and the percentage air space in a thread; the smaller the area of the filaments of the same roundness factor, the larger the air space in the thread.

From these data, curves have been plotted showing the correlation of roundness factor and specific covering power from which we can calculate the covering power of a thread in millimeters by multiplying the specific covering power for a given roundness factor by the square root of the calculated denier of the thread and dividing by 1000, and another curve showing the correlation between covering power and the percentage of air space, from which we can calculate the softness factor of a thread by dividing the percentage of air space found for a given specific covering power with the average legal titer in deniers of the filaments of the thread.

Method of Calculation

From curves showing the relationship of the net area to the legal titer and metric number (Figure 50), the roundness factor (Vr) by the Herzog method is $Vr = 127.32\ F/W^2$, where W is the width in micrometers, and F the gross cross-

sectional area in square micrometers. For viscose, cuprammonium, and nitrocellulose rayons, $Vr = 10{,}000\ T/W^2$, where T is the legal titer. For real and wild silk, $Vr = 11.12\ T/W^2$, and for acetate silk, $Vr = 12.07\ T/W^2$.

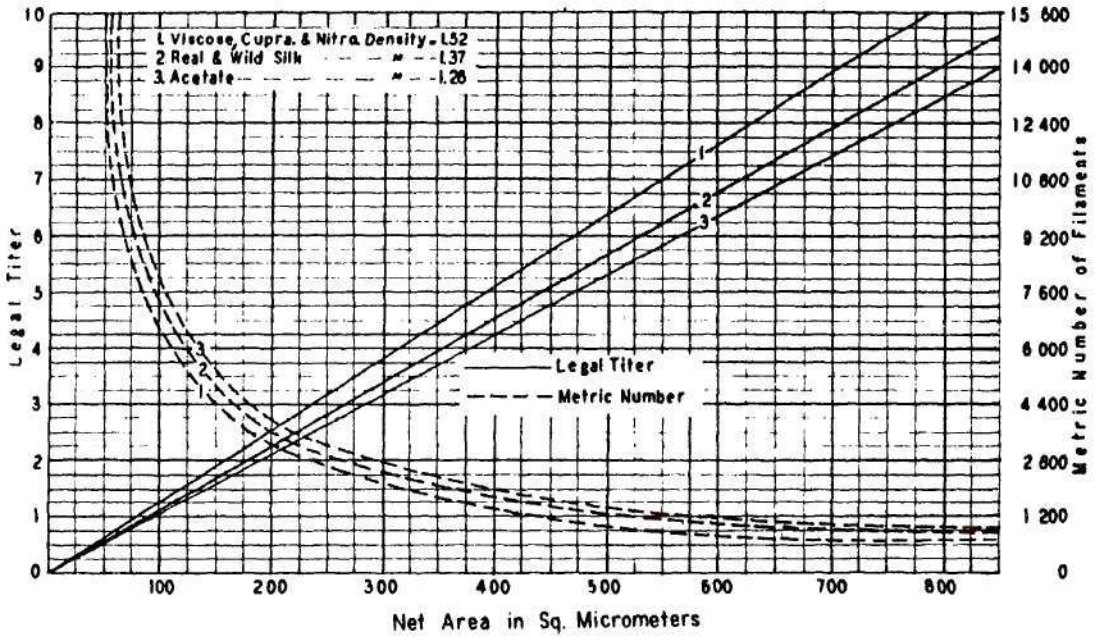

Figure 50. Relation of Net Area to Legal Titer and Metric Number

The relation of the roundness factor to the covering power of the thread (CP) and the softness factor (SF) can be calculated from the curves in Figure 51.

$CP = A\sqrt{D}/1000$ (in mm.), where A is the specific covering power in micrometers for a certain roundness factor, and D is the calculated denier of the thread.

$SF = B/T$, where B is the percentage air space and T is the legal titer in deniers of the thread.

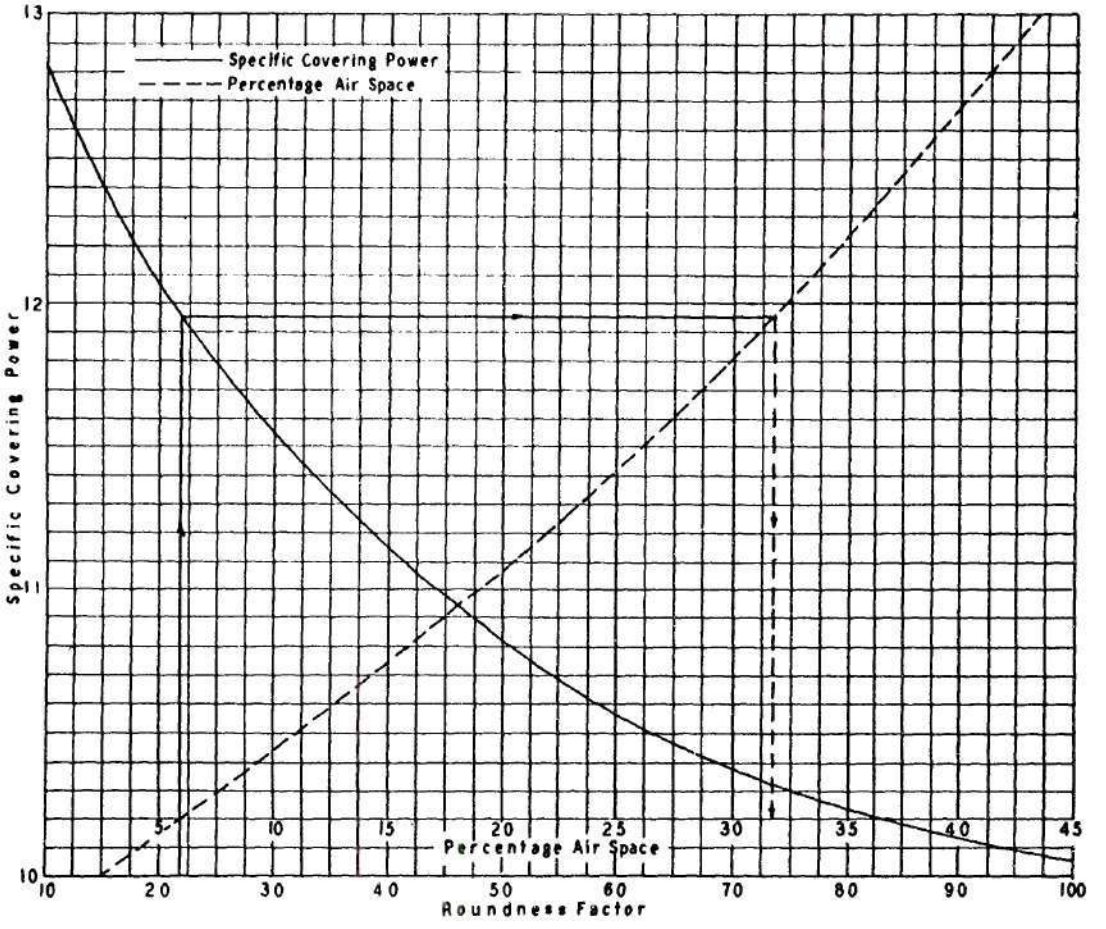

Figure 51. Relation of Roundness Factor to Covering Power and Softness Factor

All the cross-sectional data can be calculated from the measurements of only four factors (width, thickness, gross area, and net area); this is illustrated in Table XIII, the data being taken from the cross sections illustrated in Figure 52.

TABLE XIII

CROSS-SECTIONAL DATA

No.	Width, μm.	Thickness, μm.	Gross Area, $(\mu m.)^2$	Net Area, $(\mu m.)^2$
1	45	13	422	422
2	43	24	568	551
3	35	19	396	369
4	42	16	528	528
5	37	20	495	446
6	34	20	512	472
7	43	21	472	465
8	52	17	601	581
9	39	21	505	505
10	36	21	502	475
11	35	26	630	584
12	34	22	561	468
13	42	21	446	436
14	35	24	604	551
15	41	24	534	528
16	48	15	462	462
17	35	21	538	485
Total	676	325	8776	8355
Average	39.75	19.10	516.23	491.47

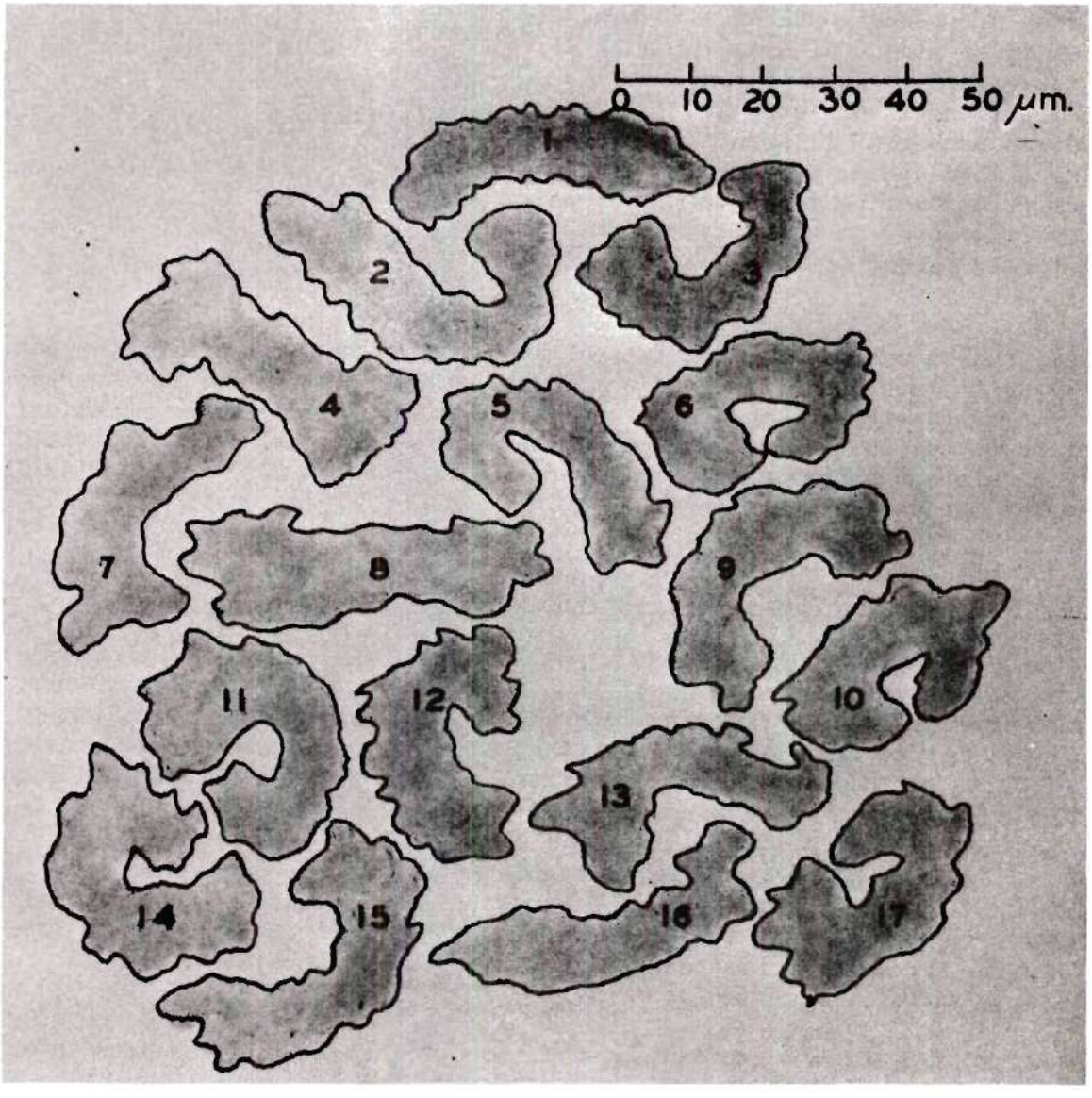

Figure 52. Cross-Sectional Data

From the data of Table XIII, the following values can be obtained.

Number of Filaments – 17

Width in micrometers	39.75
Thickness in micrometers	19.10
Width/thickness	2.31
Roundness factor	41.80
Gross cross-sectional area in $(\mu m.)^2$	516.23
Net cross-sectional area in $(\mu m.)^2$	491.47
Area of air pockets in $(\mu m.)^2$	24.76
Legal titer in deniers	6.30
Metric number of filaments	1340
Calculated denier of thread	151.20
Specific covering power in micrometers	11.47
Covering power of thread in mm.	0.14
Softness factor of thread	3.20

Kami and Nakashima (37) determined the softness factor of rayon threads by dividing the average cross-sectional area of the filaments by the average-legal titer in deniers of the filaments. This gives a much larger numerical evaluation of the softness factor but if the cross-sectional area is divided by the calculated denier of the whole thread, the results will be practically the same as the above figures.

Cross-Sectional Dimensions of Pulp Fibers (38)

Any one who has made a study of the cross sections of fibers from paper cross sections has seen that they differ considerably from cross sections of fibers seen in wood sections or other plant stems. Therefore, a more thorough study of cross sections of fibers made from pulps having various characteristics should be of interest.

A small number of fibers were straightened out and bundled together parallel with their ends overlapping at intervals, desiccated, mounted, and cross sections made as described in the chapter on microtome sectioning (Chapter XI). From the cross-section mounts, drawings were made at 1000 diameters as shown in Figure 53.

From the drawings, measurements were made of the width and thickness of the cross sections of each fiber, the perimeters of the fibers were determined by actual measurements, and the roundness factors were calculated on the basis of the relationship of the gross area of the fibers to the area of their circumscribed circles. The average of these calculations is noted in Figure 53.

Having obtained these data, the drawings of the cross sections of the fibers were then placed side by side in cordwood fashion in an arbitrarily selected unit area (163 x 84.5 micrometers) until filled. The fibers were always taken in numerical order (see Figure 53) with no regard to selection and fitting in special spaces (Figure 54).

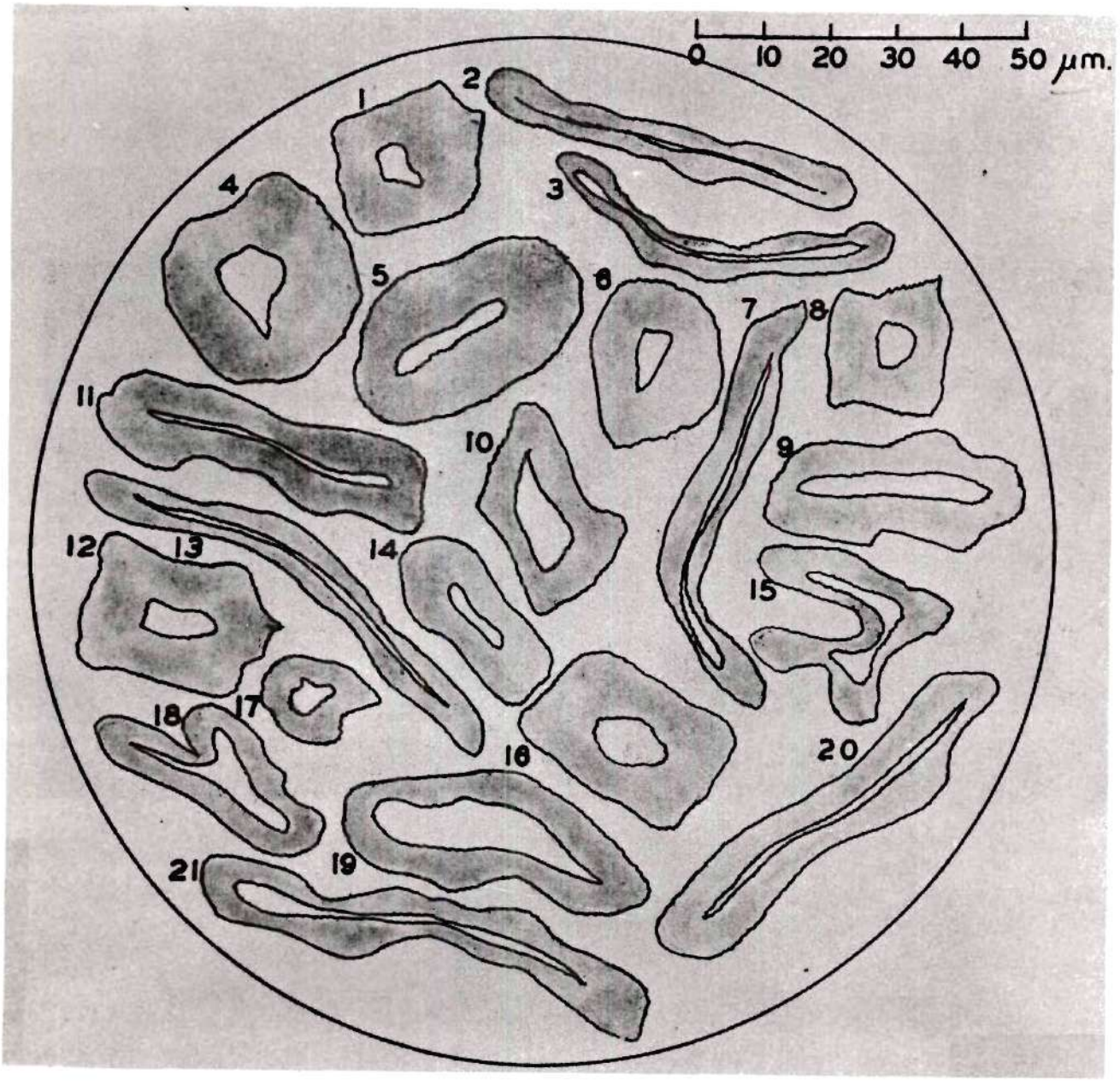

Figure 53. Douglas-fir (Pseudotsuga menziesii)
Unbleached Kraft

Averages

Width, µm.	40.00
Thickness, µm.	17.00
Width/thickness	2.35
Gross area, $(\mu m.)^2$	440.00
Area of lumen, $(\mu m.)^2$	55.00
Net area, $(\mu m.)^2$	385.00
Roundness factor	45.00
Perimeter, µm.	96.00

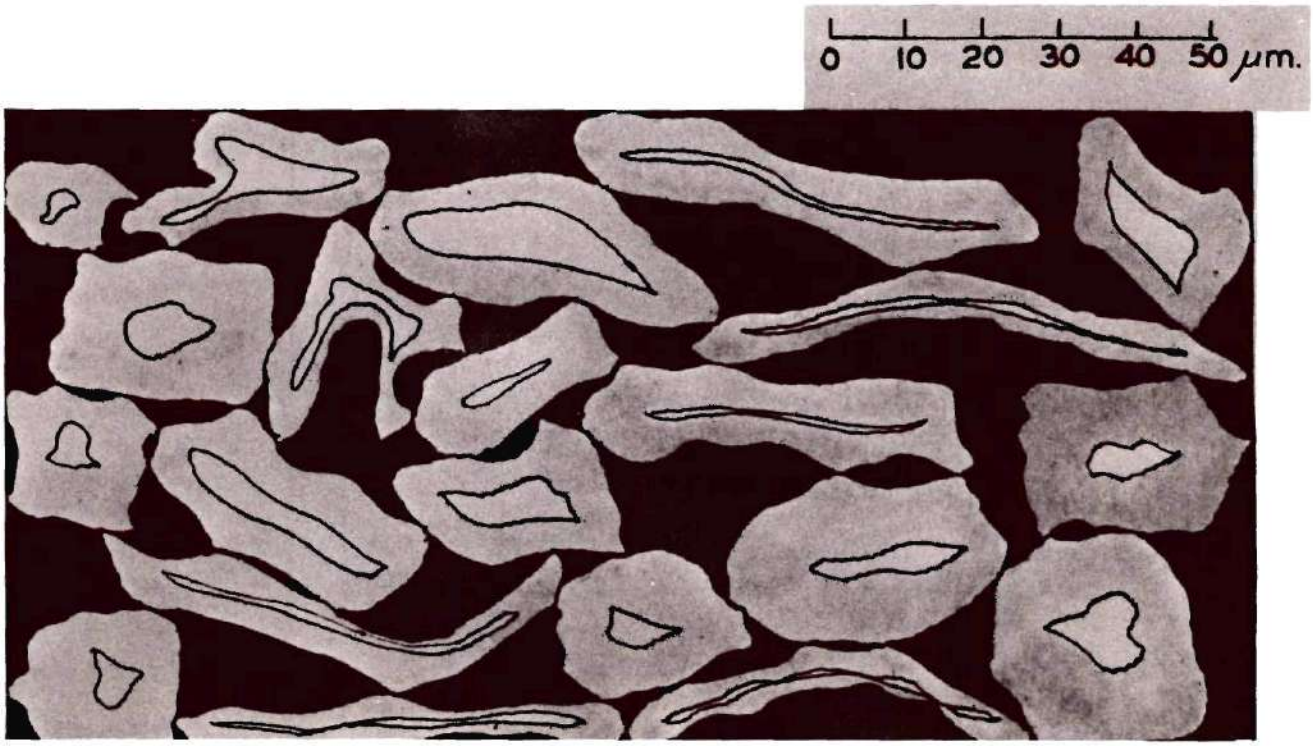

Figure 54. Douglas-fir (Pseudotsuga menziesii)
Unbleached Kraft

Averages		Unit Area Factors	
Width, μm.	40.00	Number of fibers	21
Thickness, μm.	17.00	Gross area	67.00%
Width/thickness	2.35	Area of lumen	8.40%
Gross area, $(\mu m.)^2$	440.00	Net area	58.60%
Area of lumen, $(\mu m.)^2$	55.00	Air space	33.00%
Net area, $(\mu m.)^2$	385.00	Specific surface, $(\mu m.)^2$	2057.00
Roundness factor	45.00		
Perimeter, μm.	96.00		

From the data so far obtained, it is possible to determine the number of fibers per unit area (163 x 84.5 micrometers) of each pulp and the percentage of gross and net areas of the fibers, the percentage of the area of the lumen, and the percentage of air space between the fibers in a given unit area, together with the specific surface, which is the average perimeter of the fibers multiplied by the number of fibers in a given unit area and considering the rectangle to be 1 μm. deep.

The averages calculated from the individual sections of the fibers with the unit area factors have been noted for each individual pulp represented and for the purpose of a thorough understanding of the relative importance of these data, one should make a close comparison of the tabulated data.

In former work it was found that the average width of the fibers was positively correlated with the average gross and net area of the cross-sectional area of the fibers, with the average perimeter, and with the percentage gross and net area in a given unit area, and negatively correlated with the number of fibers in a unit area, and the specific surface of the fibers; wherefore, to get a still better understanding of the importance of these data it would be well to determine the number of fibers in a given weight of pulp.

THE NUMBER OF FIBERS PER GRAM OF PULP AND ITS RELATION TO FIBER DIMENSIONS (18)

Determination of Number of Fibers

The following variables have been standardized: (1) Samples are conditioned and weighed at 50% R.H., and (2) Method of dyeing the fibers.

For the purpose of preparing strongly dyed fibers, two different methods are used:

(a) Unbleached pulp. One-half gram of pulp is soaked in 50 ml. of distilled water, rolled between the fingers, and put into a 500-ml. Erlenmeyer flask for disintegration, using the original 500 ml. of water. The beaker is rinsed with an additional 50 ml. of water, which is added to the pulp in the Erlenmeyer flask.

The pulp suspension is then heated to 50°C., 5 ml. of a 2% solution of Malachite green is added, and the suspension is gradually brought to a boil. After boiling for 10 minutes, 0.2 g. of tannic acid is added, the suspension is boiled for another 10 minutes, and water added to make one liter.

(b) Bleached pulp. The dyeing procedure for bleached pulp is the same as for unbleached, except that 10 ml. of 0.2% solution of Congo red is used instead of Malachite green and, after boiling for 10 minutes, 0.5 g. of soda ash and 5 g. of sodium sulfate is added. The suspension is then boiled for another 10 minutes, and water added to make one liter.

(3). Dilution of Suspension

For coniferous pulps, the original one-liter suspension of the dyed fibers is diluted as follows: The 1,000-ml. suspension (a in Figure 55) is divided into

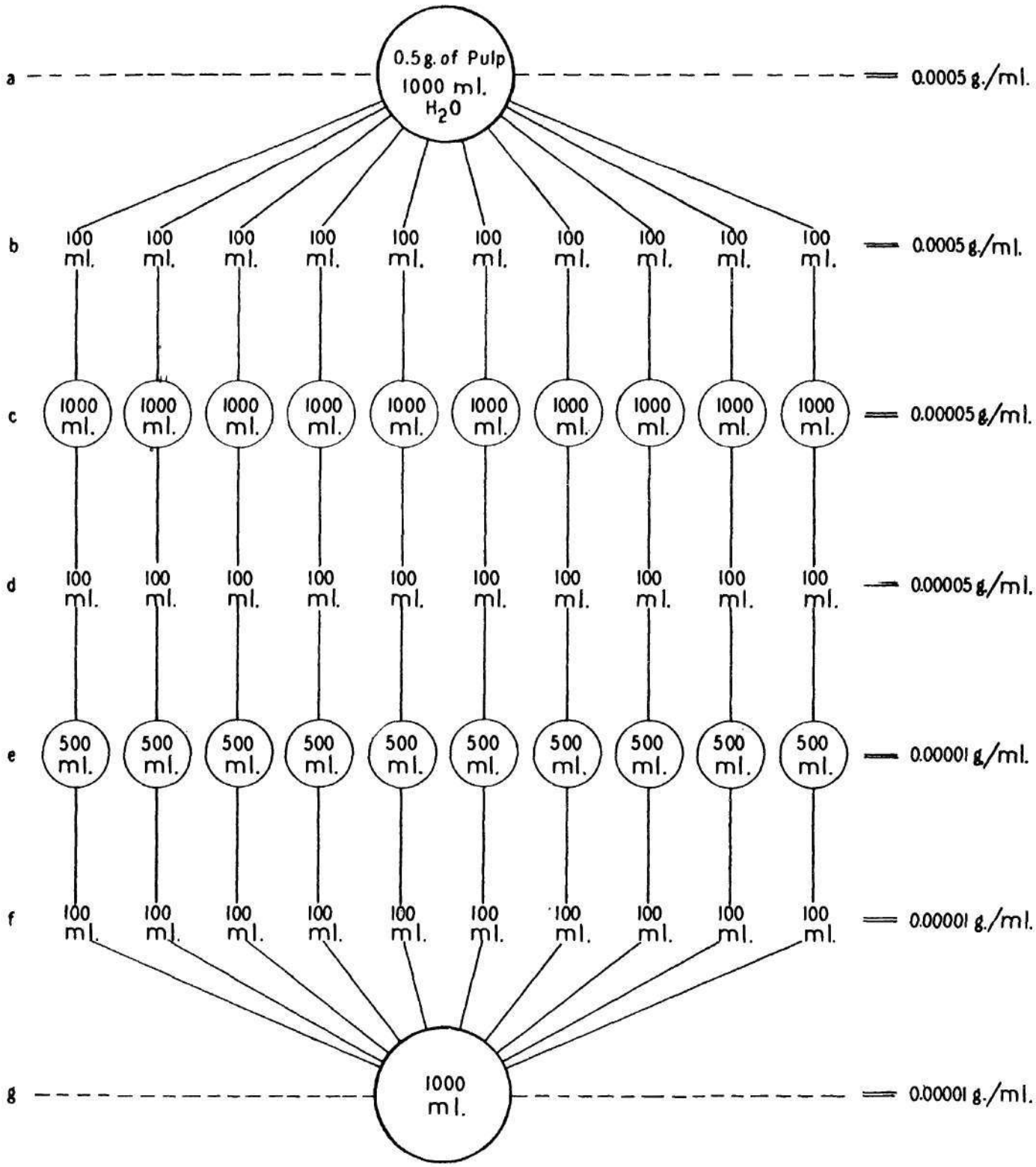

Figure 55. Dilution for Coniferous Pulps

ten 100-ml. portions (b); each one of these is diluted to one liter (c). From each of these 1000-ml. portions, 100 ml. is taken and diluted to 500 ml. (e); from each of these 500-ml. suspensions, 100 ml. are taken and combined, making a final 1000-ml. suspension containing 0.00001 g. of pulp per ml. (g).

For hardwood pulps, step (e) in Figure 55 is changed so that the dilution is 1000 ml. instead of 500 ml., giving a suspension finally of 0.000005 g. of pulp per ml. of suspension.

(4). Sampling

The graduated pipet used for sampling has an inside diameter of 7.5 mm. and is constricted at the bottom end to 5 mm.

The suspension is shaken vigorously; with one finger over the top end of the pipet, it is immediately inserted in the suspension to the depth of 11 cm. The finger is removed and 5 ml. of the suspension is permitted to rise into the pipet, the top end is closed with the finger, and the volume is transferred to a clean lantern slide cover glass 3-1/4 x 4 inches, on which a square 8.5 x 5.5 cm. has been marked with aluminum stearate.

The suspension is spread inside the square, the slide placed on a hot plate, and the fibers distributed by tapping the suspension with a dissecting needle. When the fibers are dry, the slide is covered with another lantern slide cover glass to prevent dust from settling on the slide.

(5). Counting

The lantern slide with the dried fibers is placed on a white lacquered metal plate as shown in Figure 56, and the whole is placed under a Greenough binocular microscope. Using a magnification of seven diameters, the fibers on the slide are counted. The ruled squares on the lacquered plate assist materially in making the counts.

The number of fibers in each 5-ml. portion taken from the final suspension (g in Figure 55), multiplied by 20,000, equals the number of fibers per gram of coniferous pulps, and, multiplied by 40,000, equals the number of fibers per gram of hardwood pulps.

However, as it is very difficult, at a magnification of seven diameters to differentiate between fibers less than 0.1 mm. long and dust and debris, a micrometer scale is placed in one of the eyepieces so that the fibers less than 0.1 mm. long shall not be considered in the count.

Having standardized the different steps of the procedure, let us see what is the number of fibers per gram of pulp and the relation of this value to the dimensional data, counting only fibers having a length of 0.1 mm. or longer.

The results of the counts for one of the pulps, Southern pine unbleached kraft, are presented in Table XIV.

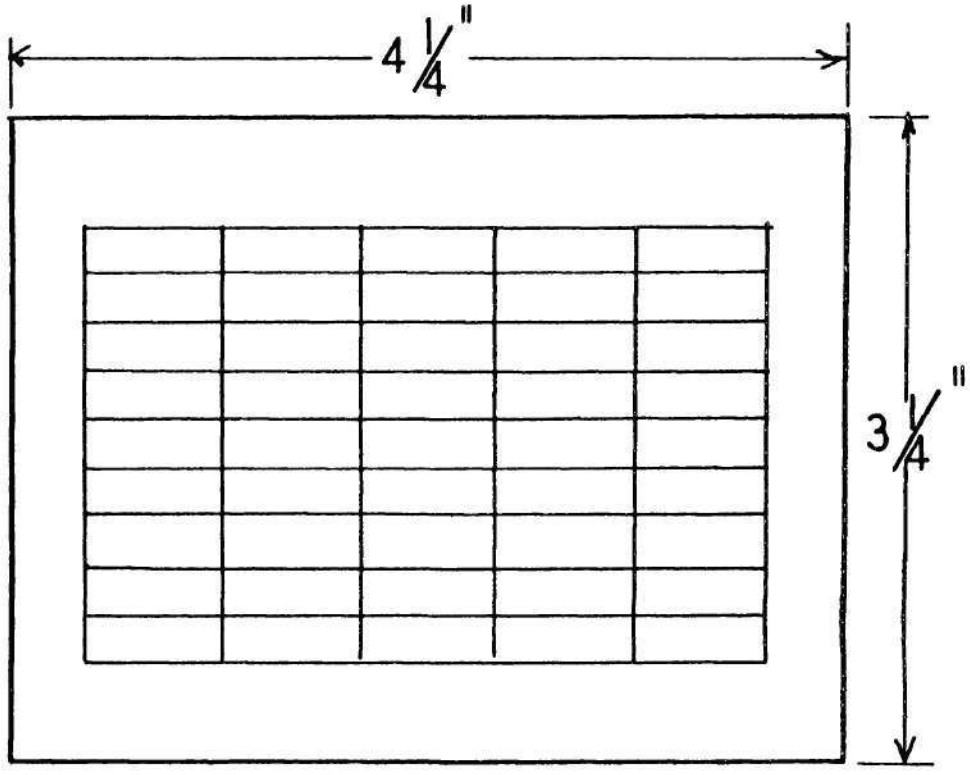

Figure 56. White Enameled Plate for Fiber Counting

TABLE XIV

SOUTHERN PINE UNBLEACHED KRAFT

Count	1	2	3	4	5	6	7	8	9	10
Number of fibers	185	210	176	198	160	173	191	193	153	193

Total number of fibers per gram of pulp = 183 x 20,000 = 3,700,000

S.D. of single counts 330,000 = 9.0%

S.D. of average 104,000 = 2.8%

The summary of the counts and standard deviations (S.D.), using ten counts of 5-ml. suspensions, is given in Table XV. This table shows that the number of fibers per gram of pulp varies considerably. This is caused, in part, by the difference in species and geographical location of the original wood; the number may also be influenced by the method of chipping, and as Lampen suggested (39), by the degree of cooking and the method of purification of the pulp fibers.

A study of the standard deviation of the counts, both for a single count and for the average of ten counts of the different pulps, shows that the method of counting is fairly accurate and dependable, even for single counts; for the average of ten counts, the standard deviations are very small for work of this kind. Therefore, if it should be desired to determine the effect of chipping, cooking, purification, or beating on the number of fibers per gram of pulp, such counts can be carried out with the assurance that the values obtained will be dependable for purposes of comparison.

(6). Time Required

The weighing, dyeing, and disintegration of a single sample takes about one hour. However, as several samples can be prepared at the same time, the average time per sample is considerably less.

The counting of a single slide requires from five to ten minutes; therefore, ten slides of a single sample should easily be counted in one or two hours.

Fiber Dimensions of the Pulps

For the purpose of determining the average fiber dimensions of each pulp, and the relationship, if any, between the fiber dimension data and fiber counts, fiber dimensions were determined for all the pulps and the number of ray cells in each of the coniferous pulps was noted. A summary of the calculated values for all these pulps is given in Table XVI.

TABLE XV

SUMMARY OF COUNTS

Pulp	Number of Fibers per g. of Pulp	Standard Deviation: Single Count, %	Standard Deviation: Average of 10 Counts, %
Southern pine unbleached kraft	3,700,000	9.0	2.8
Eastern jack pine unbleached kraft	6,700,000	5.6	1.3
Western hemlock unbleached kraft	5,500,000	5.1	1.6
Western softwood bleached sulfite	6,200,000	5.8	1.9
Softwood unbleached Mitscherlich sulfite	6,500,000	5.3	1.7
Balsam fir unbleached sulfite	8,400,000	4.4	1.3
Eastern softwood unbleached sulfite	8,700,000	7.0	2.2
Eastern softwood bleached sulfite	6,800,000	4.6	1.5
Softwood paper-grade alpha	5,600,000	5.5	1.7
Rag filter paper	9,200,000	4.8	1.5
Hardwood bleached sulfite	22,400,000	3.6	1.1
Hardwood bleached soda	22,600,000	3.9	1.2
Hardwood paper-grade alpha	22,000,000	5.0	1.5

TABLE XVI

FIBER COUNTS, WEIGHTS, AND DIMENSIONAL DATA FROM LIMIT OF VISIBILITY AND UP

Sample	No. of Fibers per Gram of Pulp	Arithmetic Average Length (a), mm.	Weighted Average Length (b), mm.	Average Width, mm.	Length (a)/ Width Ratio	Length (b)/ Width Ratio	Total Length, m.	Total Width, m.	Total Area, $dm.^2$
1	4,500,000	1.39	3.27	0.034	40.90	96.00	6,200	155	21.20
2	12,300,000	0.63	2.18	0.032	19.50	67.30	7,800	309	24.80
3	5,800,000	0.80	2.28	0.028	28.10	81.50	4,600	162	13.00
4	9,100,000	0.49	1.96	0.024	20.40	81.70	4,500	218	10.70
5	11,100,000	0.51	2.38	0.022	23.20	108.00	5,500	244	12.50
6	9,500,000	0.64	1.65	0.024	26.90	68.80	6,100	228	13.10
7	9,500,000	0.58	1.60	0.025	23.20	64.00	5,500	237	13.80
8	8,900,000	0.85	2.30	0.028	30.40	82.20	7,600	249	21.20
9	6,900,000	0.98	2.29	0.023	42.50	99.50	6,800	159	15.60
10	9,300,000	0.58	0.99	0.020	28.90	49.50	5,400	186	9.70
11	59,100,000	0.18	0.65	0.018	10.00	35.70	10,600	1060	19.20
12	38,300,000	0.33	0.69	0.024	13.70	28.75	12,600	918	30.30
13	37,900,000	0.35	0.76	0.018	19.40	42.20	12,500	682	22.50

Since only fibers longer than 0.1 mm. were counted, the actual number of fibers in a gram of pulp had to be determined from the frequency distribution of the fiber length determinations. The difference between the number of fibers counted and the number of fibers of all sizes in the pulp is shown in Table XVII.

TABLE XVII

COUNTED AND ACTUAL NUMBER OF FIBERS PER GRAM OF PULP

Name of Pulp	Counted Number of Fibers Above 0.1 mm.	Calculated Number of Fibers Above 0.0 mm.
Southern pine unbleached kraft	3,700,000	4,500,000
Eastern jack pine unbleached kraft	6,700,000	12,400,000
Western hemlock unbleached kraft	5,500,000	5,800,000
Western softwood bleached sulfite	6,200,000	9,100,000
Softwood unbleached Mitscherlich sulfite	6,500,000	11,100,000
Balsam fir unbleached sulfite	8,400,000	9,500,000
Eastern softwood unbleached sulfite	8,700,000	9,500,000
Eastern softwood bleached sulfite	6,800,000	8,900,000
Softwood paper-grade alpha	5,600,000	6,900,000
Rag filter paper	9,200,000	9,300,000
Hardwood bleached sulfite	22,400,000	59,000,000
Hardwood bleached soda	22,600,000	38,000,000
Hardwood paper-grade alpha	22,000,000	38,000,000

The following explanation will illustrate how total area shown in the last column of Table XVI is calculated for each sample.

If we wish to calculate the total projected area of the fibers in a gram of pulp (that is, the area those fibers would cover if all laid out a single layer deep), it should be noted that we cannot derive that area simply by multiplying the total length of fibers per gram by the mean width of the

fibers. Suppose we have only six fibers, which differ materially both in length and width:

	Length	Width	Area
1	70	0.3	21.0
2	20	0.7	14.0
3	4	0.3	1.2
4	50	0.5	25.0
5	25	1.2	30.0
6	60	0.2	12.0
Total	229	3.2	103.2
Mean	38.2	0.53	

The product of the total length by mean width is 122.1, an erroneous figure for total area. The product of total width by mean length gives the same erroneous figure. The correct value for total area can be obtained only by adding the individual values of area.

Although the percentage by number of fibers less than 0.1 mm. in one case is more than 60, the percentage by weight, as shown in Table XVIII, is very low in most cases and varies from 0.3 to 2.7 for coniferous pulps, is only 0.15 for the rag filter paper, and for the hardwood pulps varies from 5.4 to 16.7 (based on the weight of the total).

The bar chart in Figure 57 shows the relative length in meters per gram of the different pulps, together with the total length of the ray cells in the coniferous pulps. The relative total area which can be covered by one gram of pulp of the different samples used in this investigation is shown in Figure 58.

Although the number of fibers per gram and the average fiber dimensions of a given pulp are of value for comparative purposes, the interrelationship between the number of fibers per gram of pulp, fiber lengths and width, length/width ratios, average fiber projected area, total fiber length, and total fiber projected area is also of interest.

TABLE XVIII

PERCENTAGE OF FIBERS LESS THAN 0.1 mm. IN A GRAM OF PULP

Name of Pulp	Percentage of Fibers Less Than 0.1 mm. By Number	By Weight
Southern pine unbleached kraft	19.0	0.5
Jack pine unbleached kraft	46.0	2.5
Western hemlock unbleached kraft	5.3	0.3
Western softwood bleached sulfite	31.9	2.6
Softwood unbleached Mitscherlich sulfite	42.0	2.7
Balsam fir unbleached sulfite	11.7	0.6
Eastern softwood unbleached sulfite	8.0	0.5
Eastern softwood bleached sulfite	24.0	0.9
Softwood paper-grade alpha	18.7	0.7
Rag filter paper	1.0	0.15
Hardwood bleached sulfite	62.0	16.7
Hardwood bleached soda	41.0	5.4
Hardwood paper-grade alpha	42.0	7.2

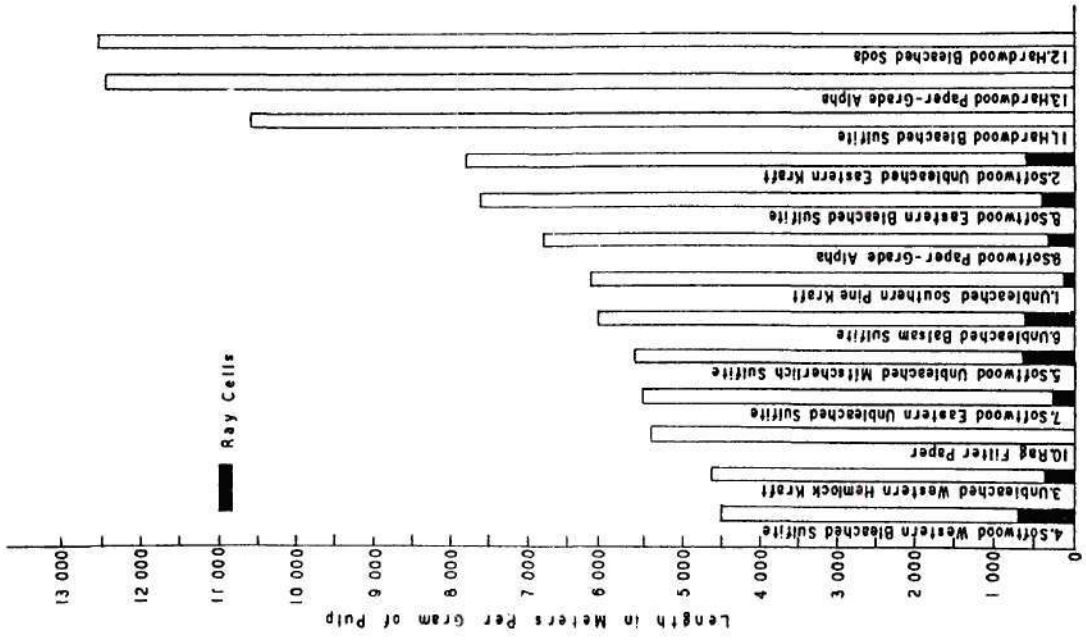

Figure 57. Total Fiber Length in Meters per Gram of Pulp

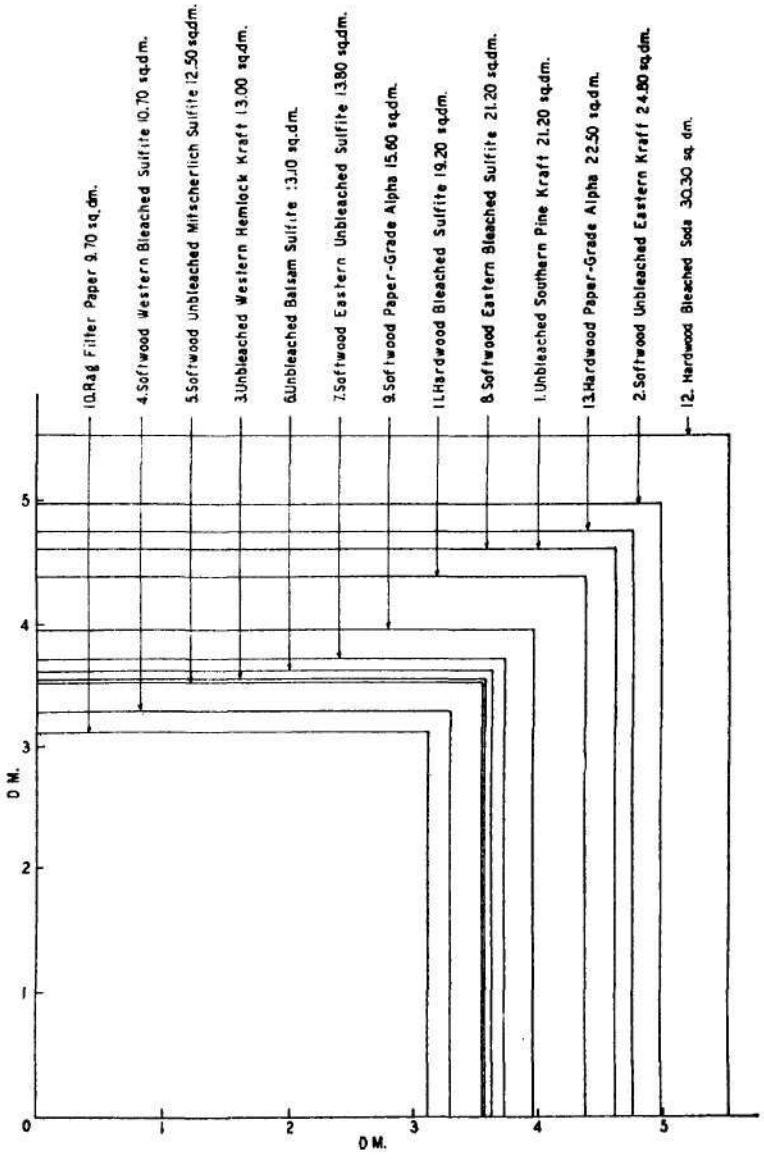

Figure 58. Total Area in Square Decimeters per Gram of Pulp

A study of the data in Table XIX reveals that the largest number of correlations and the best ones – that is, those most intimately related to the factors having a bearing upon the comparative values sought – are those obtained for fiber lengths from 0.1 mm. and longer (those included in the method used to determine number of fibers per gram of pulp). Most of these correlations are obvious. The only two which are of importance are (a) the negative correlation between the weighted average fiber length and the number of fibers per gram of pulp, -0.97; and (b) the correlation between the weighted average fiber length and the weighted length/width ratio, 1.00.

The correlation between the weighted average fiber length and the number of fibers per gram of pulp is shown in Figure 59. The importance of this correlation is the fact that, from the fiber length data, it is possible to determine directly, for a gram of a given pulp, the approximate number of fibers longer than 0.1 mm., the total length in meters, and the area of the fibers.

INTERRELATIONSHIP OF FIBER DIMENSION DATA

Having developed a relatively fast and accurate method for determining the length and width of the fibers, the number of fibers in a given weight (gram), and the ratio of these numbers to the average weighted length of the fibers, and also the cross-sectional dimensions of the pulp fibers and the ratio of these factors to a given unit area, let us take an entirely new set of pulp fibers to check the foregoing and to determine the interrelationship of all these data.

The following pulp fibers have been selected:

Softwood Pulps

1.* Southern pine (*Pinus elliottii*) unbleached kraft
2.* Jack pine (*Pinus banksiana*) unbleached kraft
3. Swedish softwood unbleached kraft
4.* Douglas-fir (*Pseudotsuga menziesii*) unbleached kraft
5.* Western hemlock (*Tsuga heterophylla*) unbleached kraft
6. Eastern hemlock (*Tsuga canadensis*) unbleached sulfite
7. Western softwood bleached sulfite
8. Balsam fir (*Abies balsamea*) unbleached sulfite
9. Scandinavian softwood unbleached sulfite
10. Softwood unbleached Mitscherlich sulfite
11. Softwood paper-grade alpha

Hardwood Pulps

12. Hardwood paper-grade alpha
13.* Hardwood bleached soda
14. Hardwood bleached sulfite

*Indicate illustrations of cross sections in text (Figure 54, 60-63, pages 98, 115-118).

TABLE XIX

CORRELATION COEFFICIENT OF INTERRELATIONSHIPS

(Only correlations above 5 times the probable error considered)

Number of Fibers per Gram of Pulp to	
Weighted av. fiber length	-0.97
Length (b)/width ratio	-0.97
Total width per gram of pulp	0.88
Arithmetic Average Fiber Length to	
Weighted av. fiber length	0.88
Length (a)/width ratio	0.88
Length (b)/width ratio	0.88
Weighted Average Fiber Length to	
Average width	0.88
Length (b)/width ratio	1.00
Average Width of Fibers to	
Length (b)/width ratio	0.88
Total Length per Gram of Pulp to	
Total area per gram of pulp	0.88

Probable error of correlation: 0.88 P.E. = ± 0.10; 0.97 P.E. = ± 0.03

Bast Fibers

15.* Kozo (Broussonettia papyrifera) from handmade tissue
16. Mitsumata (Edgeworthia papyrifera) from machine-made tissue

Leaf Fibers

17.* Abaca or manila (Musa textilis) macerated
18. Sisal (Agave sisalana) macerated
19. Caroa (Neoglaziovia variegata) macerated

Seed Hairs

20.* Cotton (Gossypium sp.) rag filter paper

*Indicate illustrations of cross sections in text (Figures 64-66, pages 119-121).

From these pulps cross-sectional dimensions were determined. The results are shown in Table XX, and the fiber length data are shown in Table XXI. The number of fibers per gram of pulp was estimated from the chart shown in Figure 59.

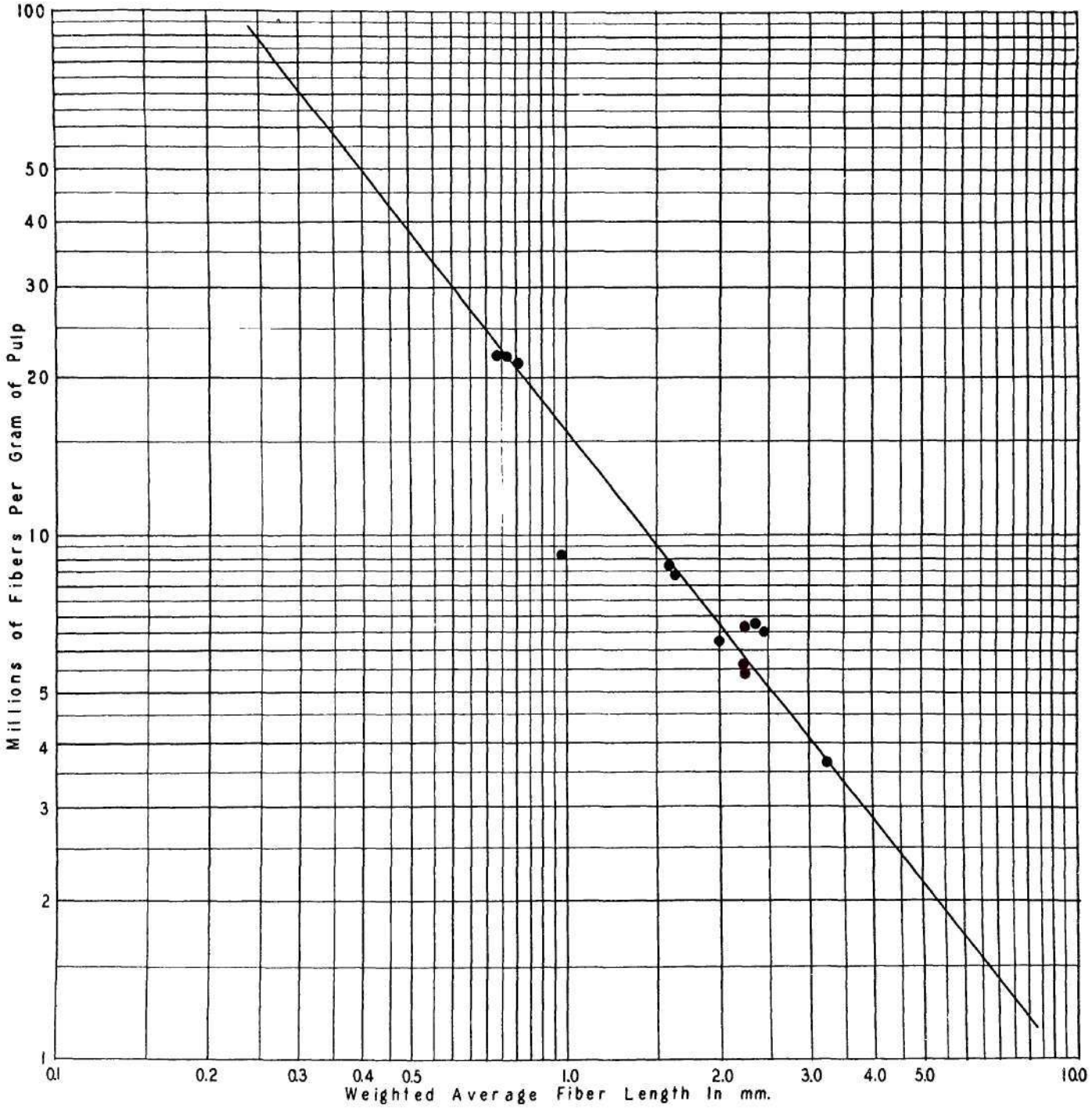

Figure 59. Correlation of Weighted Average Fiber Length from 0.1 mm., and up to Millions of Fibers per Gram of Pulp

TABLE XX

CROSS-SECTIONAL DIMENSIONS

	Averages							Unit Area Factors					
Pulps	Width, μm.	Thickness, μm.	Width/ Thickness	Gross Area, $(\mu m.)^2$	Area of Lumen, $(\mu m.)^2$	Net Area, $(\mu m.)^2$	Round-ness Factor	Peri-meter, μm.	Number of Fibers in Unit Area	Gross Area, %	Area of Lumen, %	Net Area, %	Air Space, %	Specific Surface, $(\mu m.)^2$
						Softwood Pulps								
1	40	13	3.08	364	61	303	34	98	23	61.10	10.15	50.95	38.90	2222
2	30	10	3.00	198	27	171	30	67	48	71.30	10.60	60.70	28.70	3249
3	33	8	4.03	196	21	175	28	72	53	75.40	8.05	67.35	24.60	3811
4	40	17	2.35	440	55	385	45	96	21	67.00	8.40	58.60	33.00	2057
5	31	13	2.38	267	45	222	39	81	36	69.00	11.56	57.44	31.00	2868
6	35	10	3.50	238	35	203	29	82	42	70.00	10.18	59.82	30.00	3407
7	35	10	3.50	244	22	222	32	80	39	70.00	6.15	63.85	30.00	3100
8	33	9	3.67	206	26	180	27	76	47	70.00	8.92	61.08	30.00	3566
9	29	7	4.50	152	18	134	27	61	64	70.80	8.60	62.20	29.20	3932
10	28	8	3.50	174	20	154	31	54	58	78.80	14.50	64.30	21.20	3415
11	26	8	3.24	155	21	134	32	60	68	76.60	10.10	66.50	23.40	4080
						Hardwood Pulps								
12	20	8	2.50	114	11	103	38	45	92	76.50	7.40	69.10	23.50	4113
13	21	7	3.00	97	10	87	30	43	102	71.70	7.70	64.00	28.30	4377
14	19	8	2.37	109	12	97	39	47	96	76.00	8.50	67.50	24.00	4541
						Bast Fibers								
15	18	9	2.00	136	21	115	50	43	84	82.40	11.98	70.42	17.60	3540
16	13	6	2.16	60	7	53	42	26	183	78.40	8.94	69.46	21.60	4518
						Leaf Fibers								
17	17	9	1.89	128	23	105	56	41	89	81.40	13.45	67.95	18.60	3543
18	16	10	1.60	115	18	97	59	39	93	80.30	12.16	68.14	19.70	3547
19	6	5	1.64	30	3	27	53	18	351	85.70	7.30	78.40	14.30	3796
						Seed Hairs								
20	20	10	2.00	169	21	148	50	52	66	80.80	9.94	70.86	19.20	3502

TABLE XXI

LENGTH AND WIDTH DIMENSIONS

Pulps	Arithmetic Av. Fiber Length (a), mm.	Weighted Av. Fiber Length (b), mm.	Average Width, mm.	Length (a)/Width Ratio	Length (b)/Width Ratio	Average Area, mm.^{2}	Estimated Number of Fibers per g. in Millions	Percentage by Weight from 0-0.5 mm.	0.5-2.0 mm.	2.0 and up mm.
					Softwood Pulps					
1	2.12	3.11	0.036	59	86	0.085	3.97	0.62	10.17	89.15
2	1.73	2.23	0.039	44	57	0.071	6.00	0.72	35.92	63.36
3	1.41	2.06	0.033	43	62	0.052	6.50	2.04	37.83	60.13
4	2.21	3.63	0.039	57	93	0.104	3.32	0.83	8.96	90.21
5	2.21	2.80	0.041	54	68	0.100	4.50	0.71	17.34	81.95
6	1.97	2.93	0.039	51	75	0.082	4.20	1.45	19.52	79.03
7	0.49	1.96	0.024	20	82	0.012	6.90	13.54	35.83	50.63
8	1.73	2.25	0.040	43	56	0.074	5.75	1.25	27.17	71.58
9	1.17	1.93	0.032	37	60	0.042	7.00	4.78	39.42	55.80
10	0.51	2.38	0.022	23	108	0.012	5.35	8.07	17.68	74.25
11	0.98	2.29	0.023	43	100	0.023	6.90	4.81	30.21	64.98
					Hardwood Pulps					
12	0.35	0.76	0.018	19	42	0.006	37.90	23.09	76.91	0.00
13	0.33	0.69	0.024	14	29	0.008	38.30	35.41	64.59	0.00
14	0.18	0.65	0.018	10	36	0.003	59.10	46.73	53.27	0.00
					Bast Fibers					
15	5.44	6.63	0.022	247	301	0.121	15.50	0.00	2.41	97.59
16	2.73	3.18	0.015	182	212	0.044	3.80	0.00	11.78	88.22
					Leaf Fibers					
17	3.60	4.06	0.017	218	239	0.063	2.70	0.00	3.45	96.55
18	2.64	3.08	0.014	189	220	0.039	3.95	0.00	16.04	83.96
19	2.29	2.69	0.007	327	384	0.016	4.68	1.01	12.45	86.54
					Seed Hairs					
20	0.58	0.99	0.020	29	50	0.012	16.00	28.78	62.25	8.07

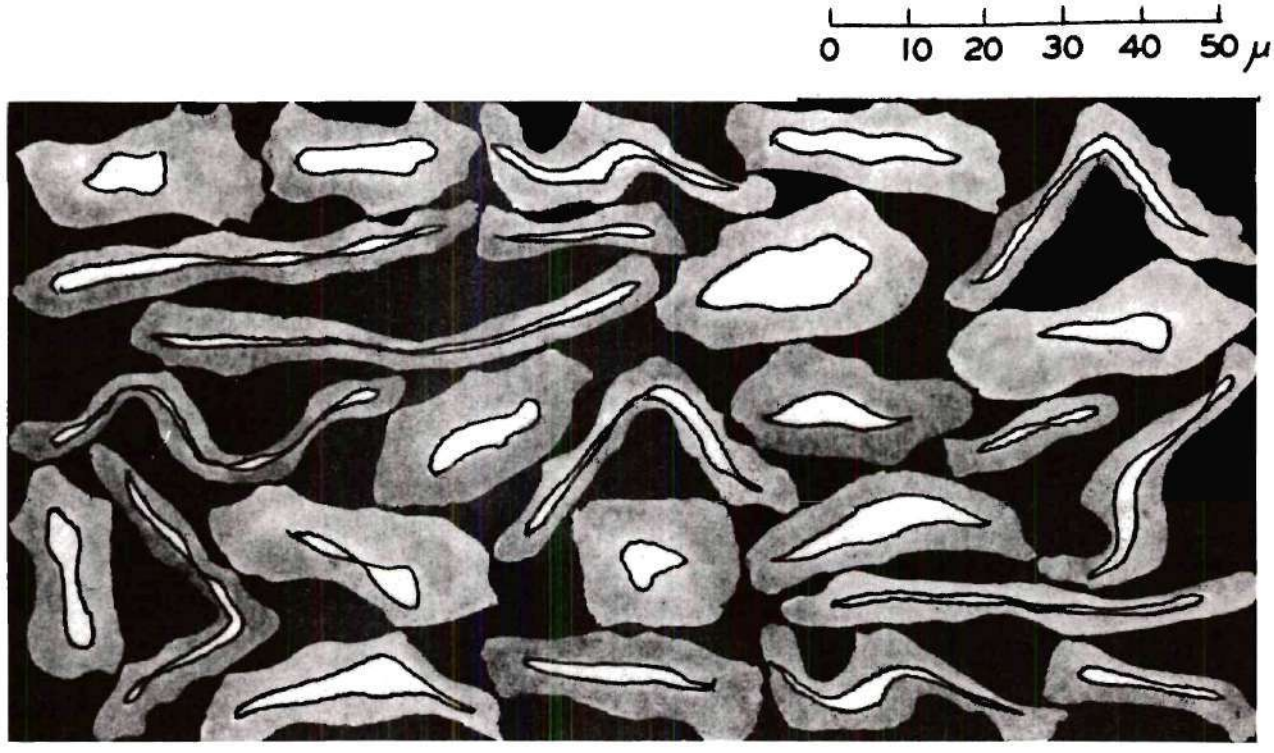

Figure 60. Southern Pine (Pinus elliottii)

Unbleached Kraft

Averages		Unit Area Factors	
Width, μm.	40.00	Number of fibers	23
Thickness, μm.	13.00	Gross Area	61.10%
Width/thickness	3.08	Area of lumen	10.15%
Gross area, $(\mu m.)^2$	364.00	Net area	50.95%
Area of lumen, $(\mu m.)^2$	61.00	Air space	38.90%
Net area $(\mu m.)^2$	303.00	Specific surface, $(\mu m.)^2$	2222.00
Roundness factor	34.00		
Perimeter, μm.	98.00		

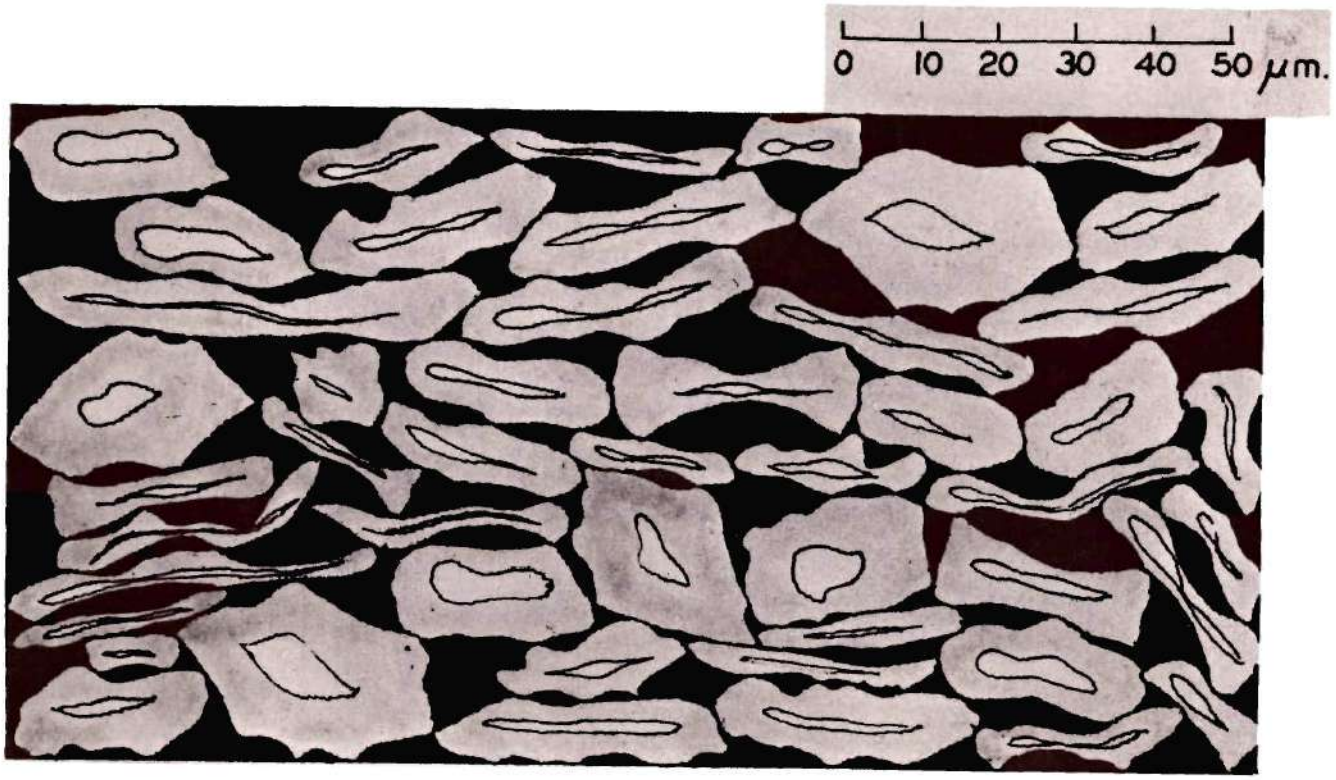

Figure 61. Jack Pine (Pinus banksiana)

Unbleached Kraft

Averages	
Width, μm.	30.00
Thickness, μm.	10.00
Width/thickness	3.00
Gross area, $(\mu m.)^2$	198.00
Area of lumen, $(\mu m.)^2$	27.00
Net area, $(\mu m.)^2$	171.00
Roundness factor	30.00
Perimeter, μm.	67.00

Unit Area Factors	
Number of fibers	48
Gross area	71.30%
Area of lumen	10.60%
Net area	60.70%
Air space	28.70%
Specific surface, $(\mu m.)^2$	3249.00

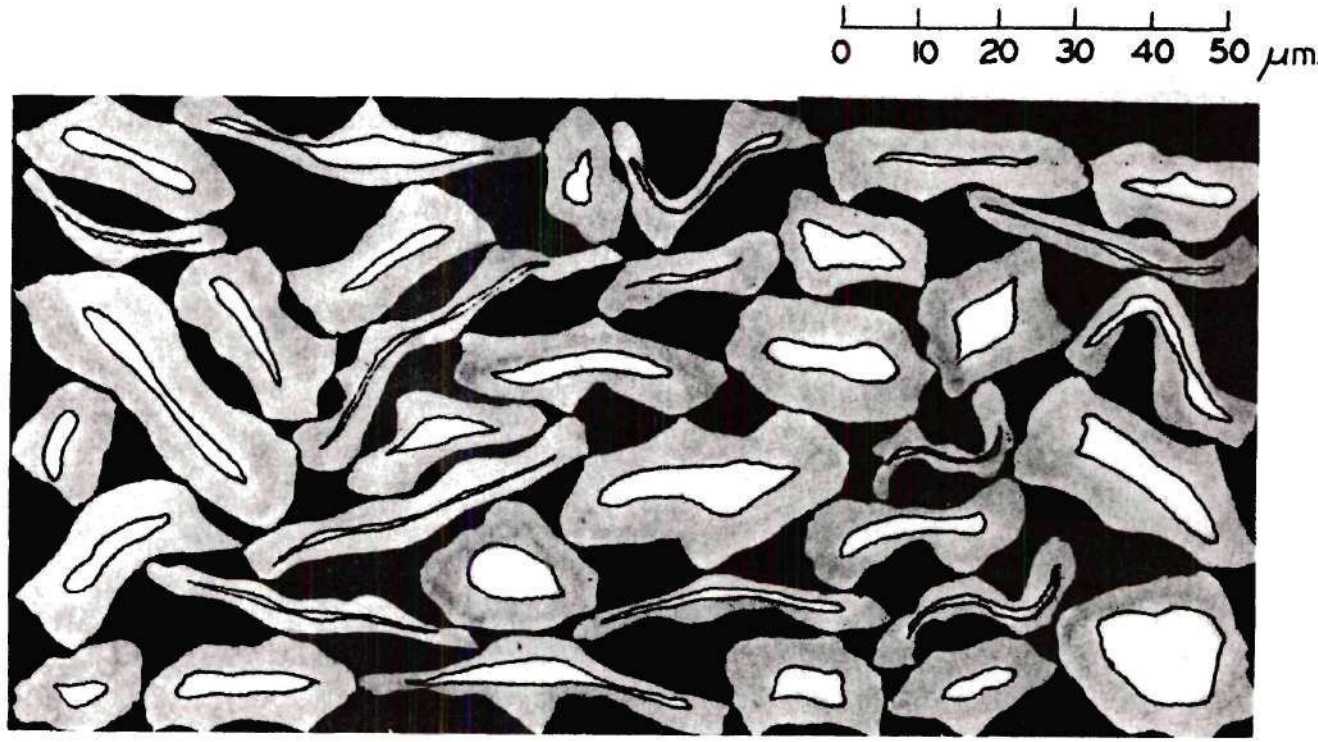

Figure 62. Western Hemlock (Tsuga heterophylla)

Unbleached Kraft

Averages	
Width, μm.	31.00
Thickness, μm.	13.00
Width/thickness	2.38
Gross area, $(\mu m.)^2$	267.00
Area of lumen, $(\mu m.)^2$	45.00
Net area, $(\mu m.)^2$	222.00
Roundness factor	39.00
Perimeter, μm.	81.00

Unit Area Factors	
Number of fibers	36
Gross area	69.00%
Area of lumen	11.56%
Net area	57.44%
Air space	31.00%
Specific surface, $(\mu m.)^2$	2868.00

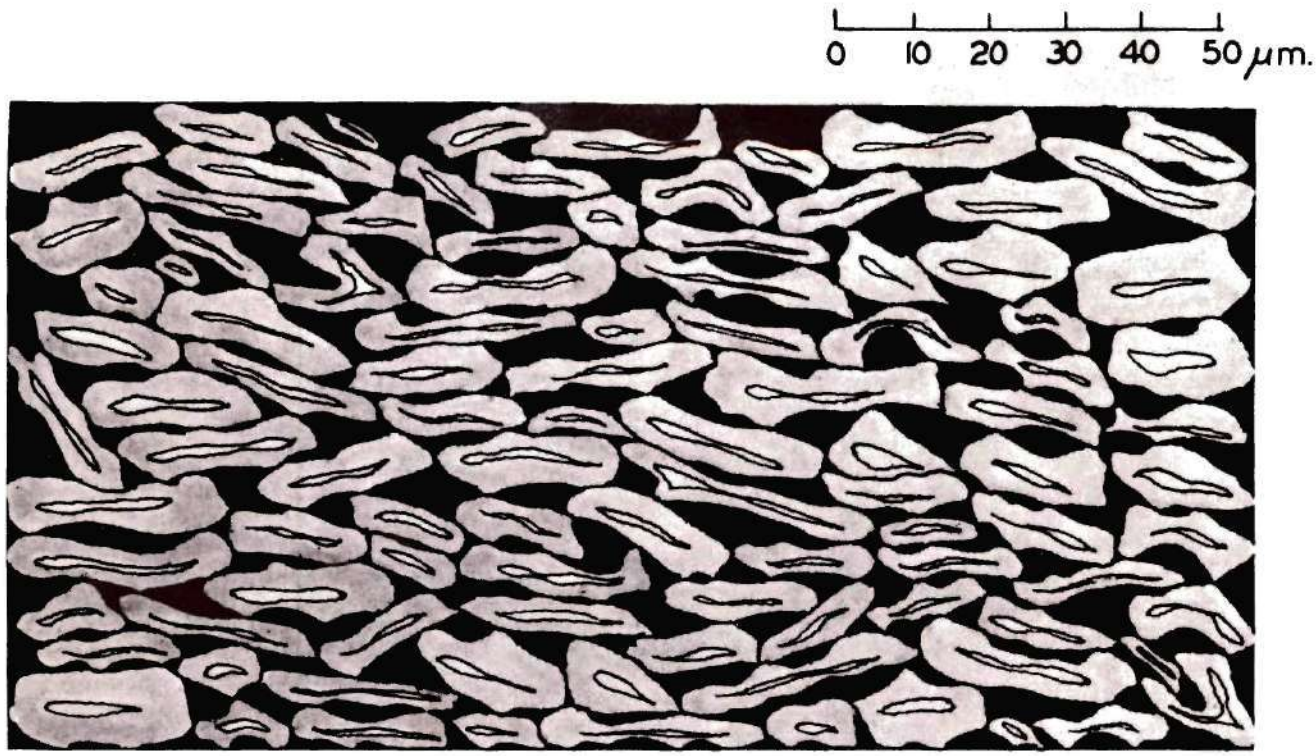

Figure 63. Hardwood Bleached Soda

Averages	
Width, μm.	21.00
Thickness, μm.	7.00
Width/thickness	3.00
Gross area, $(\mu m.)^2$	97.00
Area of lumen, $(\mu m.)^2$	10.00
Net area, $(\mu m.)^2$	87.00
Roundness factor	30.00
Perimeter, μm.	43.00

Unit Area Factors	
Number of fibers	102
Gross area	71.70%
Area of lumen	7.70%
Net area	64.00%
Air space	28.30%
Specific surface, $(\mu m.)^2$	4377.00

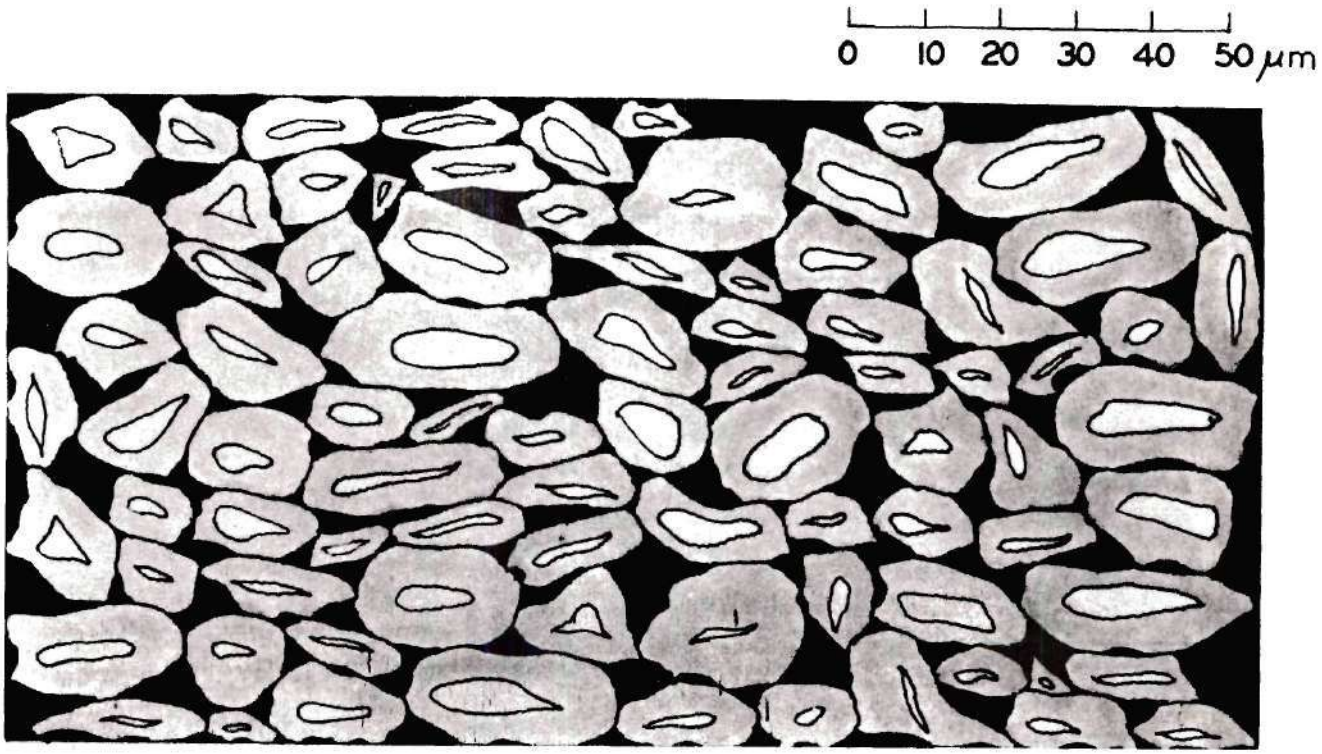

Figure 64. Kozo (Broussonettia papyrifera)

From Handmade Tissue

Averages		Unit Area Factors	
Width, μm.	18.00	Number of fibers	84
Thickness, μm.	9.00	Gross area	82.40%
Width/thickness	2.00	Area of lumen	11.98%
Gross area, $(\mu m.)^2$	136.00	Net area	70.42%
Area of lumen, $(\mu m.)^2$	21.00	Air space	17.60%
Net area, $(\mu m.)^2$	115.00	Specific surface, $(\mu m.)^2$	3540.00
Roundness factor	50.00		
Perimeter, μm.	43.00		

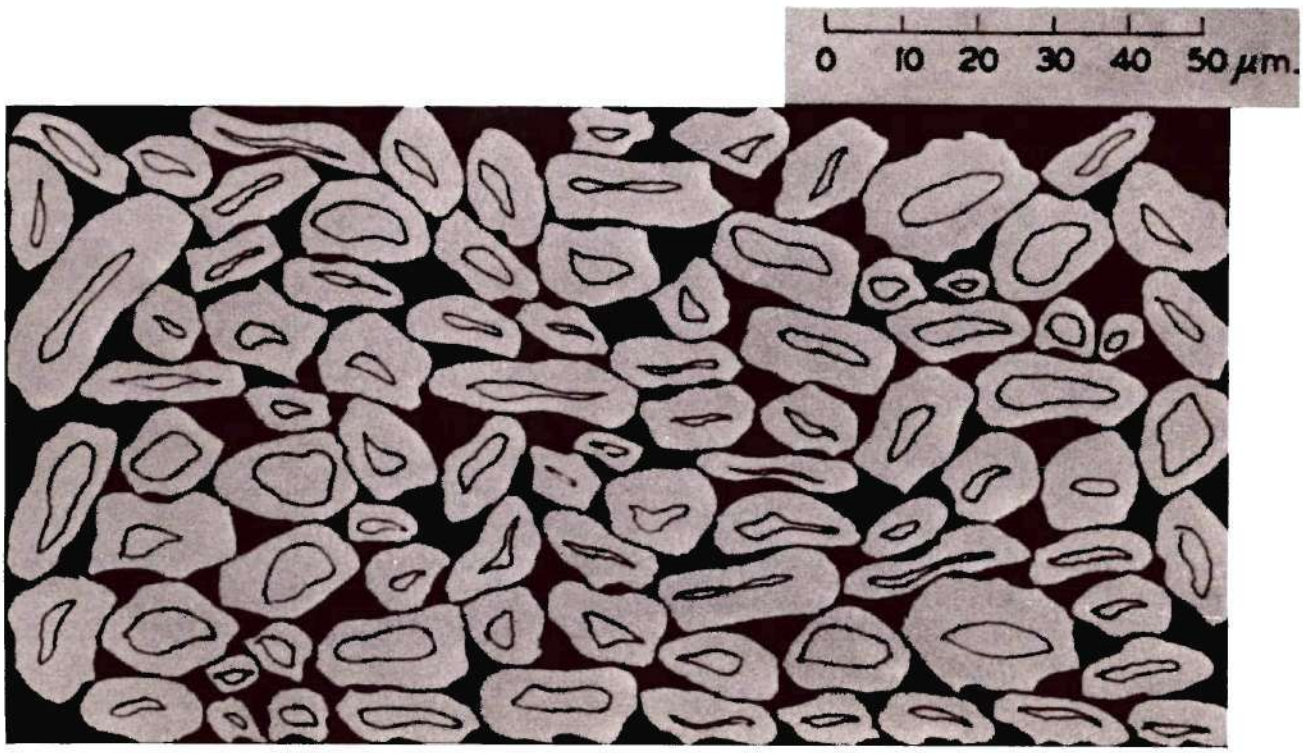

Figure 65. Abaca or Manila (Musa textilis)

Macerated

Averages	
Width, μm.	17.00
Thickness, μm.	9.00
Width/thickness	1.89
Gross area, $(\mu m.)^2$	128.00
Area of lumen $(\mu m.)^2$	23.00
Net area, $(\mu m.)^2$	105.00
Roundness factor	56.00
Perimeter, μm.	41.00

Unit Area Factors	
Number of fibers	89
Gross area	81.40%
Area of lumen	13.45%
Net area	67.95%
Air space	18.60%
Specific surface, $(\mu m.)^2$	3543.00

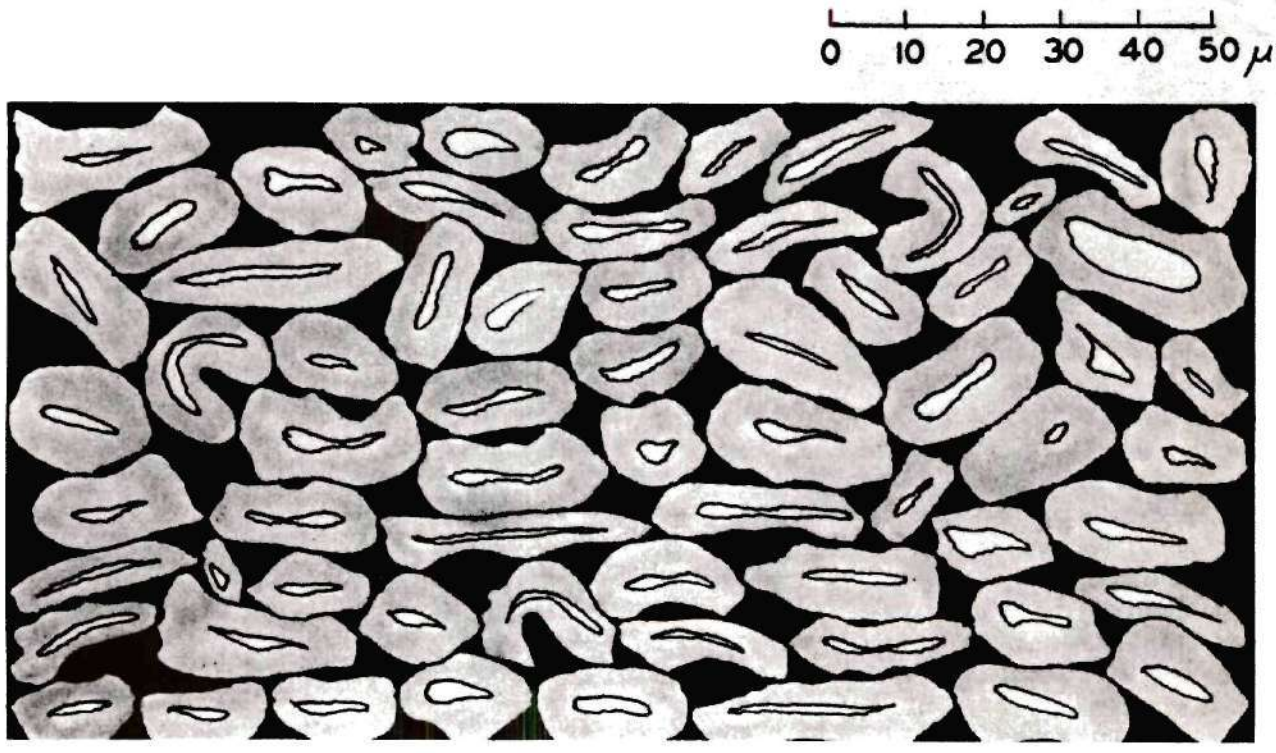

Figure 66. Cotton (Gossypium sp.)

Rag Filter Paper

Averages		Unit Area Factors	
Width, μm.	20.00	Number of fibers	66
Thickness, μm.	10.00	Gross area	80.80%
Width/thickness	2.00	Area of lumen	9.94%
Gross area, $(\mu m.)^2$	169.00	Net area	70.86%
Area of lumen, $(\mu m.)^2$	21.00	Air space	19.20%
Net area, $(\mu m.)^2$	148.00	Specific surface, $(\mu m.)^2$	3502.00
Roundness factor	50.00		
Perimeter, μm.	52.00		

Plotting the relationship between the data in these tables it was found that there was a positive correlation between the average width of the cross sections and the average gross and net area of same, and the percentage of air space between the fibers, as well as a negative correlation between the average width to the number of fibers per unit area and the percentage gross and net area.

These relationships (Figures 67-70) are of importance and also check with results of former work (38).

As already mentioned, it has been shown that the number of fibers per gram of pulp can be determined from the weighted average fiber length of the pulps and, since the width of the cross sections is the same as the width of the fibers, when accurately measured by the fiber length projection method, it is possible, although more work should be done to check this supposition, to estimate fairly accurately from these data alone, the average cross-sectional area of the fibers, the relative percentage of fibers and air space in a given unit area of the fibers in a given weight of pulp without having to make cross sections and cross-sectional calculations.

In other words, the fiber length data obtained by the projection method and the correlation charts shown in Figures 59, 67-70 suggest that further practical investigations should be made in the mills to see if, from such data, it should be possible to obtain a fairly accurate idea of the beating and felting characteristics, the surface area exposed during sizing or dyeing, and the relative air space available for the retention of fillers, etc., for a pulp.

If all or some of these suppositions should prove true, a number of mills could then not afford not to make continuous fiber length studies of their pulps and furnishes.

The practical importance of such data has been shown by Schur and McMurtrie in an article on nitration of wood pulp (40) where they state the following:

"Data obtained in the examination of a variety of coniferous wood pulps indicate that the behavior of a pulp in the acid wringer and of the beaten nitrocellulose in the poacher can be predicted from a determination of the number of fibers of pulp per unit volume of test sheet; the lower the number, the more suitable the pulp.

The beaten nitrocelluloses lend themselves more readily to dehydration in the press the lower the number of fibers per gram of untreated pulp."

FIBER COARSENESS

Coarseness is defined as the weight per unit length of fiber, expressed as mg. per 100 m. and called a decigrex (dg.). It is 10.0 times the value of the "grex" proposed as the International Standard Denier, and 11.1 times the value of the common denier used for textile fibers (p. 90 and 91). TAPPI method T 234 su-64 (Coarseness of Pulp Fibers by Projection) is a method by

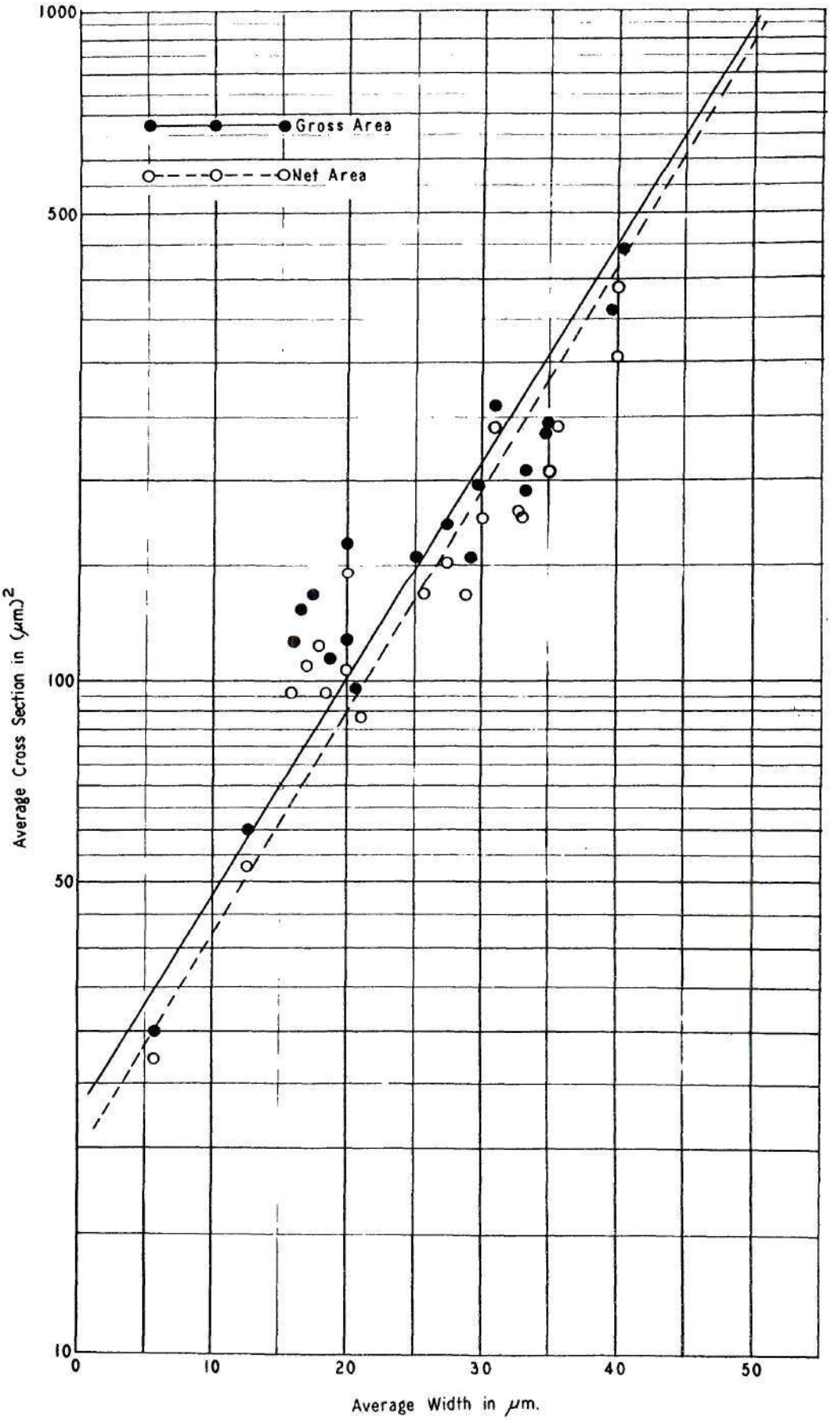

Figure 67. Correlation of Average Fiber Width to Average Gross and Net Gross Area of the Fibers

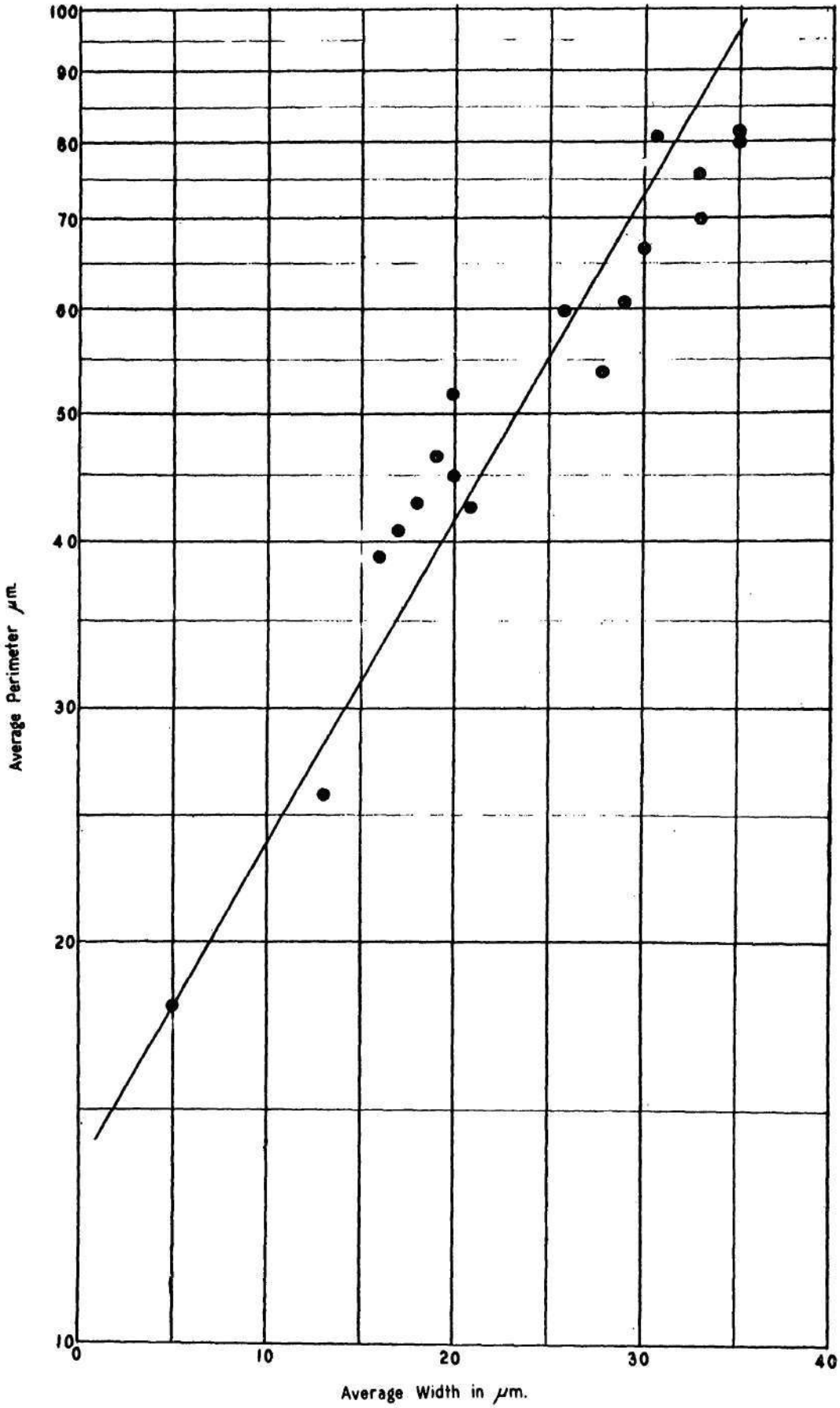

Figure 68. Correlation of Average Fiber Width to Average Perimeter

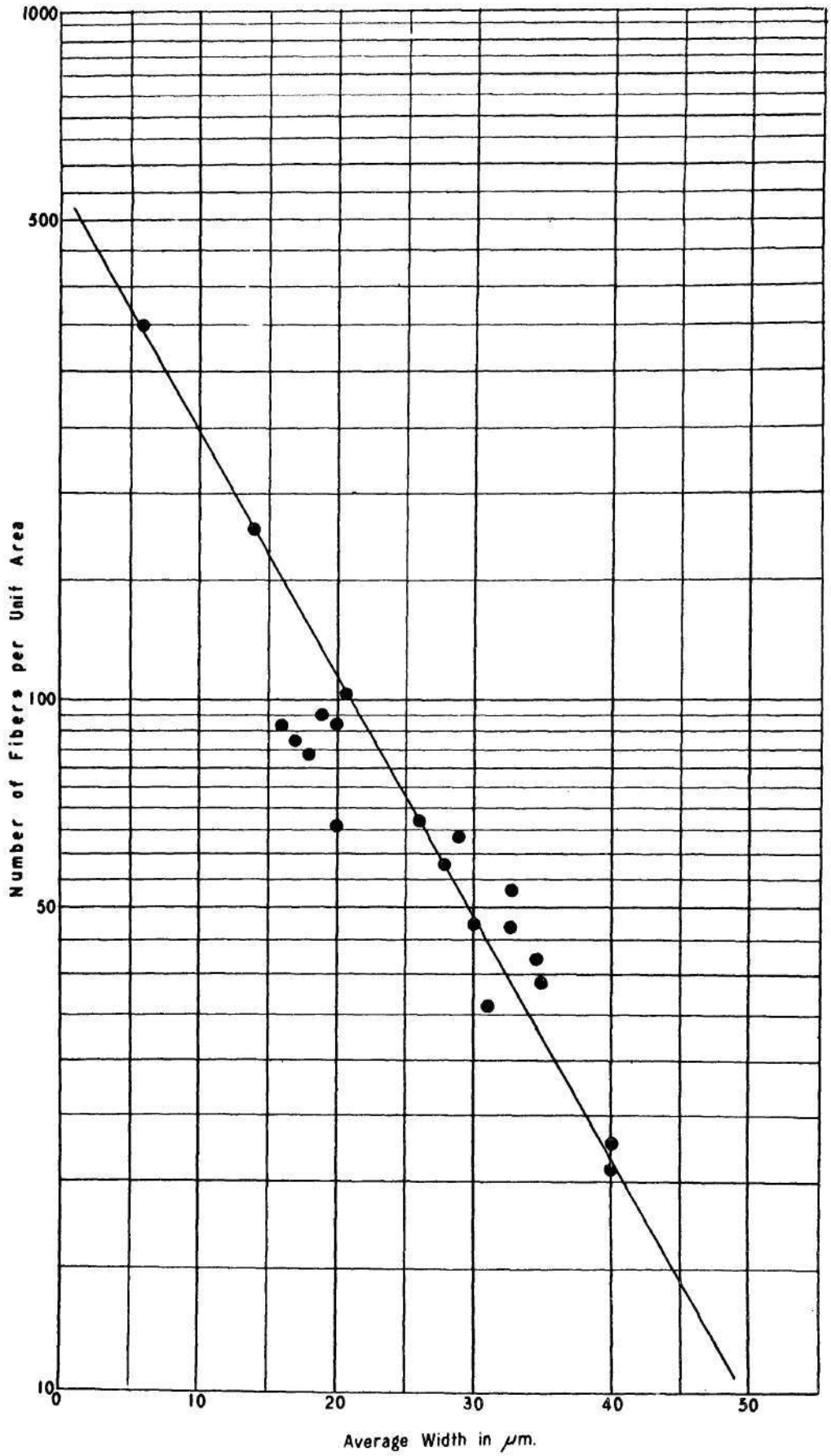

Figure 69. Correlation of Average Fiber Width to Number of Fibers per Unit Area

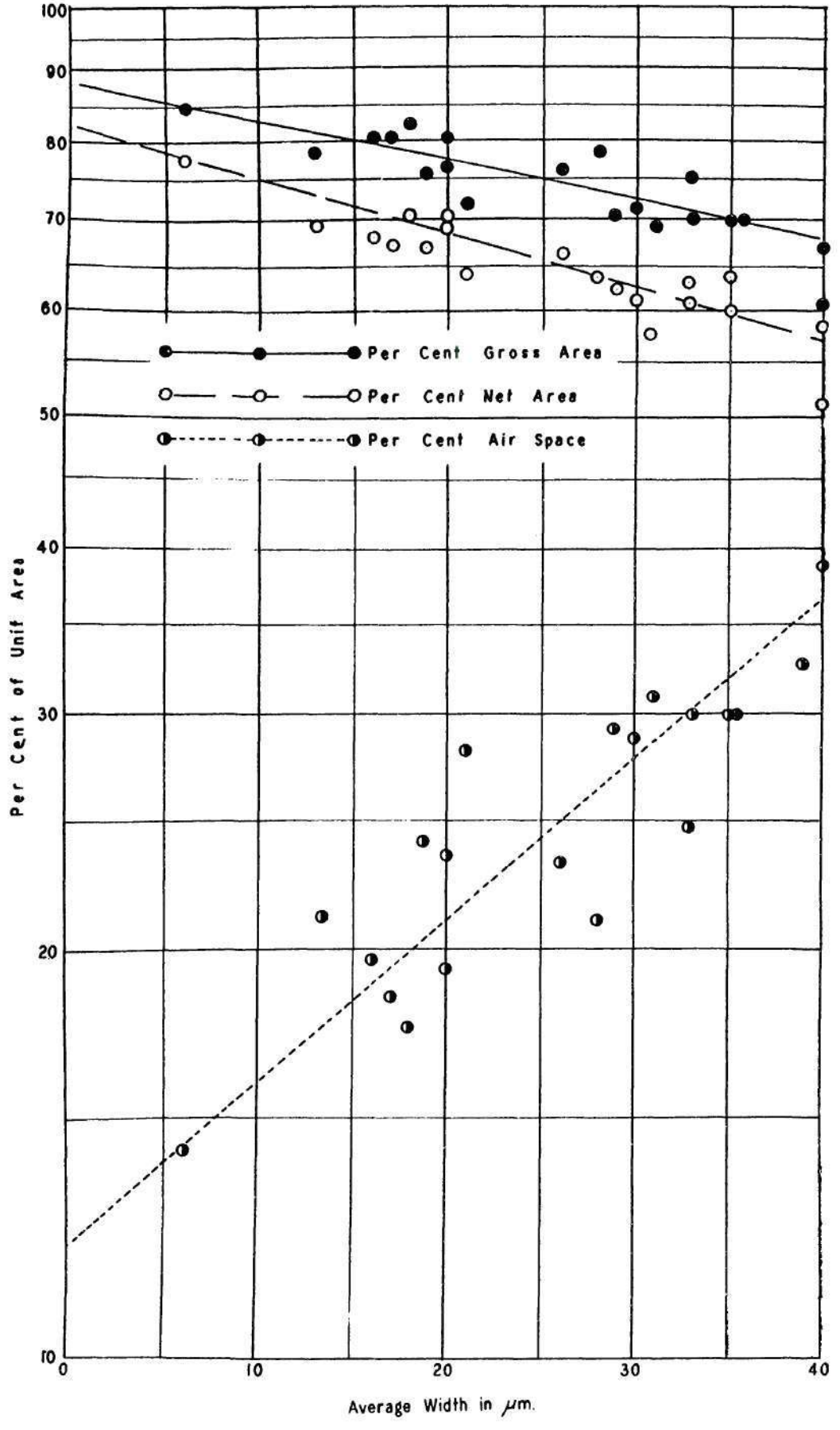

Figure 70. Correlation of Average Width to Percent Gross and Net Area and Percent Air Space

which the weight per unit length of the fibers in a pulp may be determined by counting the crossings of a known weight of fiber per unit area on a prepared slide, when their images are projected on a screen having lines of a definite length. Alternatively, if desired, the crossings may be counted with a microscope and mechanical stage, by traversing the slide through fixed distances. The units, as determined by this method, and the weight factors described in Chapter VIII (p. 236) should be proportionate. The coarseness value of a pulp should be a useful one because it includes the effects of fiber thickness, the size of the fiber lumen, and the density of the cell wall substance (13, 31, 63-66, 73).

STANDARD TECHNIQUES FOR MEASURING FIBER BENDING

Thus far only the actual length of the straightened fibers has been considered. A series of articles by Kilpper discusses the numerical evaluation of the bending factors of fibers (41).

If, for example, the kind of deviation of the fibers from the ideal shape is observed, it is easy to place them under the following classifications: 1. Curved fibers, 2. Broken fibers, 3. Twisted fibers.

Curved fibers are such as are curved instead of having a linear shape; broken fibers are fibers which at breaking points have separated into a number of linear pieces; and twisted fibers are those which although linear, are twisted along their longitudinal axis. Quite often individual fibers will have two or all of these characteristics but one of them will be predominant.

A measure of the fiber breaks, although laborious, can be determined by counting the breaking points in a given amount of fibers. The twisting can also be determined by counting the number of twisted fibers in a given amount of pulp. On the other hand, the numerical evaluation of the many-formed curvatures of the fibers becomes considerably more difficult. The course of the fiber axis does not follow any recognizable algebraic curve, and the radius of curvature changes from point to point, often discontinuously. In spite of the difficulty, the determination of the curvature stands out as the most important identification characteristic of fiber deformations.

However, this problem becomes simplified the moment the insistence on the exact geometrical curvature is abandoned and instead the measurement of the apparent shortening of the fiber extension is considered. That is, the curves themselves are not measured, but rather their total result on the fiber shape. The apparent shortening of the fiber length caused by the bending can without question be expressed by the relationship of the actual curved length to the linear distance between its end points. (The end points are the boundaries of the greatest extension between any two parts of the curved fiber. The end points, as shown in Figure 71 G, will not necessarily coincide with the fiber ends.)

The visible advantage of this method is shown in Figure 71 A to F from some exact definitions of geometric curve drawings. A shows a straight line where the real length and the linear distance between the end points is the same, and the relation of one to the other equals 1. B shows a half circle with diameter 1. Here the relationship between the real and the shortened length is $\pi/2$ or 1.57; in a complete circle C the relationship equals π, and in D the advantage of this method is particularly clear. In both figures the radius has purely geometric consideration, but even so it is evident that the fiber in Figure 71 C is bent more than the fiber in D. This is clearly shown by the relative shortening of the lengths; in the case of C the ratio is 3.14, while for D it is only 1.57. Even more pronounced is this advantage shown by comparing Figure 71 E and F.

This relationship is also a measure which gives numerical evaluation to the effect of every kind of fiber bending on the deviation from the linear extension of the fiber. By this explicit and limited description of the effect of the deviation from the linear, the same effect can be obtained from unlimited bending combinations of the same fiber. It is also recognized that using this method will necessarily collide with the folding (sharp bend) idea, because by the sharp bending the longitudinal stretch of the fiber will also be shortened. For exact description, measure of the length relations must, to a certain extent, be accompanied by a report of the specific folding points,

specific bending turning point number, and a specific twisting number. By an arithmetic combination of these four numerical values, the microscopical fiber characteristics can then, even though many-sided in reality, be expressed by a single number. Also, without expanding the measurements of the fiber conditions by all of these individual methods, the determination of the relationship of the real to the apparent fiber length is an important step forward for a numerical evaluation of the fiber form.

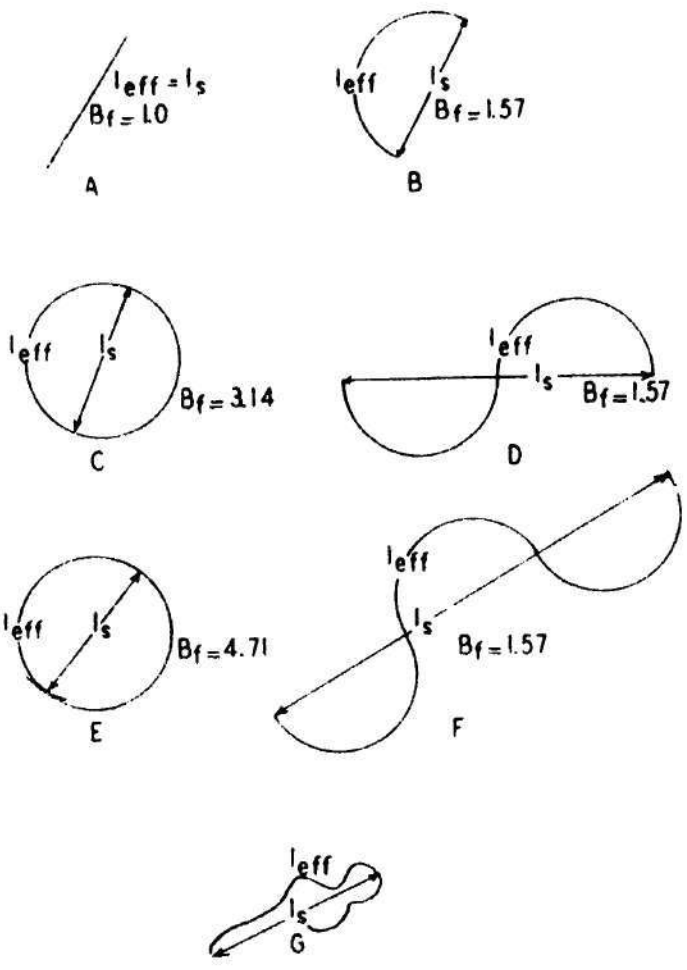

Figure 71. Diagrammatic Explanation of Bending Factors

The bending factor is the ratio of the real fiber length to the linear distance of the greatest fiber spread (Figure 71 C). This relation can be expressed by the following equation: $B_f = l_{eff}/l_s$ where B_f = the fiber bending factor, l_{eff} = the real fiber length, and l_s = the greatest linear fiber spread.

The value of B_f can therefore never be less than 1; the larger this value is the greater the deformation of the fiber. The value of B_f expresses the extent of the deformation, but gives no idea of the quality of the bending. If to the value B_f were added measurements of the folding nicks, turning points and twisting, this shortcoming could be overcome. It would then be possible to differentiate quantitatively between various fiber bending factors.

To be sure of comparative and reproducible results, the measurements of the bent fibers must be accurately made.

A number of different bent and twisted fibers with their calculated bending factors are illustrated in Figure 72.

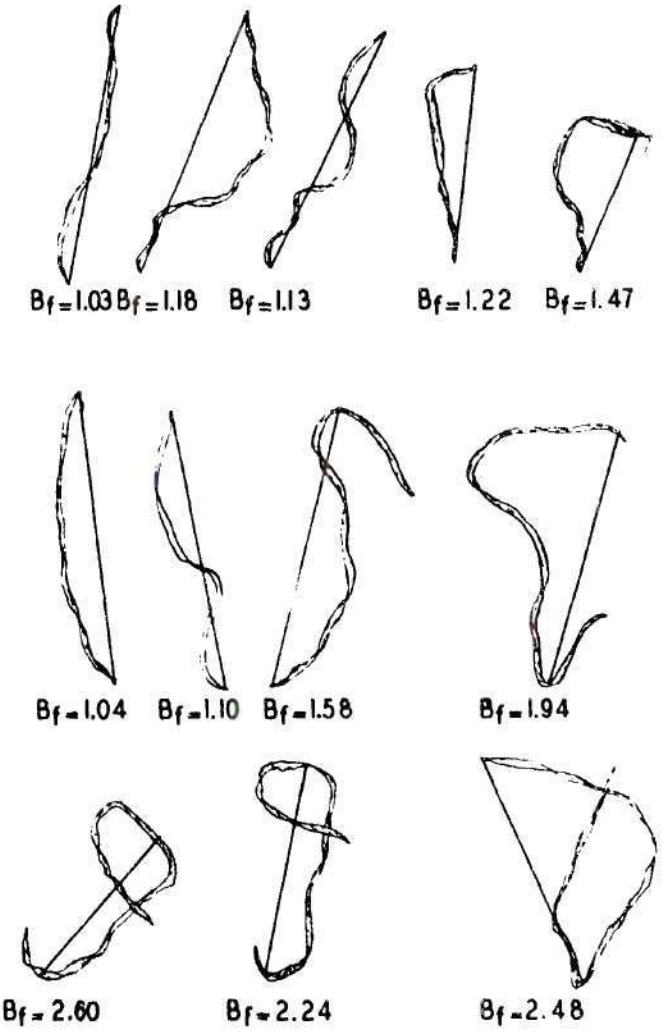

Figure 72. Bending Factors of Pulp Fibers

Summary of Results

It was not possible to find any exact relationship between the bending factors and the physical and technological characteristics of the finished paper, although it was shown that there was a tendency for a high bending factor to accompany highly purified pulp, low mechanical strength, high bulk, and high absorbency.

The investigation of the effect of beating showed that the bending factor increased during the early part of beating, but was quickly reduced as the beating pressure was increased. For the hard normal pulps, the bending factor does not increase during or after long beating. However, the bending characteristics of highly beaten pulp are qualitatively quite different from the same in unbeaten pulps.

Comparing the average bending factors for the different fiber length fractions of a pulp, it was shown that the typical differences of the bending characteristics of the fibers from purified and normal pulps not only was characteristic for the long fiber length fractions, but that the short fiber length fractions also did show this typical difference of the bending factor characteristics.

A further study of the statistical data of the fiber forms showed that it is possible from the relationship of the percentages of highly bent fibers to the average bending factor to get an idea of the character of the highly purified pulps.

SIEVE TESTING

The Bureau of Standards has found by experience that in testing sieves for conformity to the "Standard Specification for Sieves," the most reliable results are obtained by measuring the wire diameters and by determining the number of wires per centimeter, and then computing the opening by the formula:

$$\underline{O} = \frac{10}{N} - W$$

where $\underline{O}$ = average opening in mm., $\underline{N}$ = number of wires per cm., and $\underline{W}$ = average diameter of the wires in mm.

This method is also very advantageous for determining the mesh opening in other wires and similar products.

For a detailed discussion of sieving, the reader is referred to books by Cadle (67), Orr and Dalla Valle (68), and Irani and Callis (61).

PARTICLE SIZE DETERMINATION

METHODS OF DETERMINING GRAIN SIZE (42)

Ordinary screen analysis of powders is of little value except for determining the proportions of coarse grains; it gives no idea of the true average size of the grains. The finest screens procurable have 300 to 350 meshes to the inch with openings of 40 to 50 micrometers.

Microscopic study affords the most positive method of testing finely divided material which is beyond the range of standard sieves. Not only is the size of the particles measured, but shape, uniformity, aggregation, and other important properties, which affect the ultimate usefulness of the material, are observed directly. Various nonmicroscopic methods are available for particle size determinations, but they depend for confirmation upon microscopic studies, and should never be used to the exclusion of direct examinations.

Two methods are available for the microscopic measurement and count of the grains of a filler: First, accurate measurements of the diameters of a comparatively few grains with the filar micrometer eyepiece; second, the less accurate estimation of a very large number of grains in a large number of fields.

For measuring a material of uniform size in which the grains are comparatively large (5 micrometers or more) the first method probably has advantages. The second method was proposed and used in principle by Perrott and Kinney (43) and others.

PREPARATION OF SAMPLE

Depending upon the character of the material, the sample can be prepared by taking 20 grams and plunging it in a Dazy churn with one liter of distilled water for one hour with enough ammonium hydroxide to deflocculate the particles (42). Then dilute to two liters and transfer to a tall jar five inches in diameter and twelve inches high and agitate by blowing air into it through a tube which extends to the bottom of the jar. During this agitation several samples, enough to make 50 ml., are withdrawn with a pipet, and placed in a 1 x 10-inch test tube for microscopic count.

For fine abrasive powders Jones (44) suggests the following method: The dry powder is sampled carefully, the samples are reduced in size by quartering, and a small metal cup is used to pick up enough material to form a layer on the slide with few particles touching or overlapping. A clean glass slide is placed on a horizontal surface and covered with a brass tube 8.75 cm. in diameter and 50-cm. long. The top of the tube is closed with a rubber stopper through which projects the lower end of a glass tube, whose upper portion is bent to the shape of a sink trap. The sample of abrasive is placed in the bend of the trap and a sharp puff of air from a rubber bulb forces the fine powder into the upper portion of the brass tube. The grains settle through the air in the tube, forming an evenly distributed layer on the glass microscope slide. The amount

of sample is chosen so that the particles do not touch or overlap to any great extent. Two slides are prepared and protected under a watch glass until they are placed on the mechanical stage of the microscope. From ten to twenty fields are selected at random on each slide and the diameter of the grains measured. The distribution of the different sizes of grains on the slide has been found to be very uniform. Drying the original sample in a crucible heated over a flame will overcome the tendency of very fine powders to collect in clumps and aggregates.

If the material is very nonuniform in size it sometimes is necessary to separate the sample into different fractions either by different forms of air separation or, for materials insoluble in water, by elutriation.

PREPARATION OF SLIDES

The sample is suspended in distilled water in a test tube and shaken; water is added and the surplus is removed until the desired turbidity, which may be described as a cloudy or very slightly milky appearance, is obtained. The tube should not be more than one-third full. A pipet is run to the bottom and the contents are agitated by blowing air through the tube; then a few drops are quickly drawn into the pipet and placed upon the slide. The suspension on the slide should cover an area of about 20 mm. in diameter. The slide is carefully placed in an air bath and dried at 105°C. Drying on a hot plate is unsatisfactory because an uneven film results. The density should be such that all grains are separated from each other and may be counted without confusion. In general, it has been found that, except with clays, better dispersion of the particles is obtained by the use of distilled water alone than with any deflocculation agent. A drop or two of ammonium hydroxide added to the test tube will increase the dispersion of the particles of most clay samples.

TYPE OF MOUNTS

The type of mount to be used depends upon the physical properties of the particles. It is better, as a rule, to use an uncovered slide for all fillers, except those (such as clays) which have a large proportion of the grains less than one micrometer in diameter. Objectives corrected for an uncovered slide should be used. Where grains less than one micrometer in diameter must be counted, a 1.9 or 2.0-mm. oil-immersion objective is necessary and a cover glass must be employed.

Glycerin is found to be a satisfactory mounting material for many of the nonmetallic materials. Its index of refraction is 1.47 and it is viscous enough to hold the grains stationary.

Combination dispersion and mounting media will be described under the section – Preparation of Slides of Pigments and Inerts.

MICROSCOPIC EQUIPMENT

The resolving power of the optical system used is an important factor when determining size of particles in the subsieve range less than ten micrometers in diameter. The resolving power is dependent upon the NA of the optical system and the wavelength of the illuminant employed. The ordinary Abbé condenser is unsuited for work of this kind since it is undercorrected for spherical and chromatic aberration, and although the normal numerical aperture is of the order of 1.2 its aplanatic cone does not exceed 0.45.

The microscope used should be a standard research microscope with mechanical stage and both monocular and binocular eyepiece tubes. The objectives should be apochromatic and the condenser a 1.40 NA aplanatic or achromatic.

In determining the size of particles in the 0.5-5.0 micrometer range it is essential that the light be monochromatic, and Fairs (45) obtained the best results with sodium vapor light with a mean wavelength of 589 nm. Fairs (72) also used a projection microscope for particle-size analysis.

COUNT AND MEASUREMENT

Using a petrographic microscope or a microscope with polarization attachment employing crossed nicols, one can distinguish easily between floccules and individual grains; without nicols the distinction at times may be difficult.

The eyepiece is fitted with a micrometer disk ruled in squares. One of the smaller squares is again subdivided. Observing the field through this screen and knowing the value of the divisions, one can approximate closely the size of the individual grains.

Beginning with the lowest power objective and at one edge of the slide, the field is surveyed, the largest grain found and estimated, and called the even size nearest which it falls; that is, if it averages 47 μm., it is called 50 μm. Starting at the upper left corner of the 25 central squares, the field is gone over square by square and all grains that fall between 45 and 55 μm. are counted. The count is recorded as so many grains of 50 μm. diameter. Beginning again, the grains between 35 and 45 μm. are counted and recorded as 40 μm., and so on to the smallest size that can be determined accurately with the lowest power objective. The slide is then moved to a new field and the process repeated. In this way the whole slide is worked over and as many fields counted as necessary. Often the finer grains will be concentrated near the edges of the slide, and overcounting or undercounting these areas must be avoided.

The objective is then changed to the next higher power and the groups of sizes selected as falling best within its range are counted, and so on, to the highest power objective and the finest grains. In counting 1 μm. grains with the 4-mm. objective and 0.5 μm. grains with the 1.9- or 2-mm. objective, the task becomes laborious if the entire field is counted; therefore, if the sizes are well distributed in any one field, only one-fifth of the area is counted as follows: beginning at the upper left corner, the diagonal row of squares equal to one-fifth of the field are counted and the result is multiplied

by five. In the next field the diagonal line is taken from right to left, and so on.

Depending upon the relative size and character of the material under investigation, it may sometimes be advantageous to use one or the other forms of micrometer disks as shown in Figure 73.

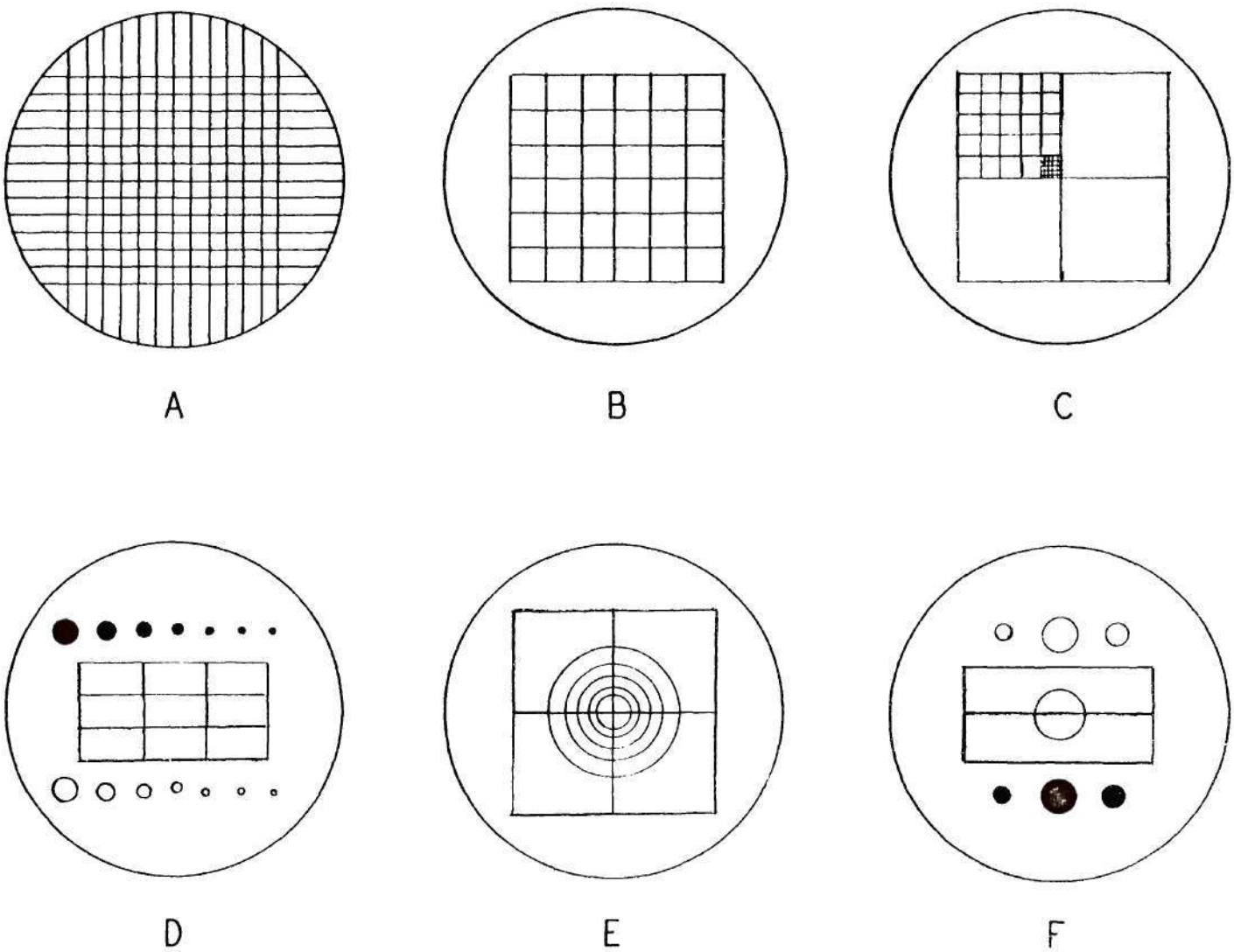

Figure 73. Types of Eyepiece Micrometer Disks (45)

As no two grains, even of the same material, have the same shape, it is necessary to average the dimensions. For example, if a grain is 38 μm. long and 22 μm. wide, it is called a 30-μm. grain.

Such an estimate assumes that the thickness of the grains equals the average observed diameter. For minerals of distinctly tabular or platey form, such as talc or mica, however, it becomes necessary to apply a correction, as the thickness of the grains that result from pulverizing is 50% or less of the average observed diameter (61, 67, 68). The shape factor may at times cause considerable difficulty in particle measurement (68, 69).

MAGNIFICATION USED AND COMPARISON OF FIELDS

For coarser grains (30 μm. or larger) a 16-mm. objective and a 10X ocular can be used. For grains of intermediate size (10 to 30 μm.) an 8-mm. objective should be used, giving a magnification of 210X. For finer grains, a 4-mm. objective, giving a magnification of 430X, is used for counting the grains from one to 10 μm. For grains less than a micrometer, a 1.9-mm. objective giving a magnification of 950X should be used. Magnifications up to 7500 diameters can be used with the projection microscope with very good definitions.

In some of the very important pigments and fillers a substantial proportion is much finer than 0.5 μm., and the usefulness depends largely on how much of that extremely fine material is present – because the surface per unit weight becomes enormous at those small sizes. In this range examination with the light microscope has definite limitations due to fuzzy diffraction patterns. Fortunately, in the electron microscope we have a tool which is extremely useful for determining particle size in this range (70). Even the electron microscope barely reaches enough resolving power for some powders at 75,000 to 100,000X. Some work has also been done on turbidimetric methods for evaluating these fine powders. Measurement of the mass of helium adsorbed on the material at low temperature and pressure has also been used to calculate specific surface directly (61, 67, 68).

AVERAGE PARTICLE SIZE

The average particle size or grain size should be expressed as a numerical value that has a definite meaning in relation to some physical property. The numerical or arithmetical mean, obtained by dividing the summation of the product of the number of each size and its diameter by the total number, has no physical significance. The average particle size should be based on surface or volume, depending upon which of these is the more important in the work for which the material is to be used.

Calculation of Average Particle Size

If one particle only is considered with its three dimensions, respectively, equal to l, b, and t, various "average diameters" may be calculated in the following ways:

$$(1)\ (l + b)/2;\quad (2)\ (l + b + t)/3;\quad (3)\ \sqrt{lb}$$

$$(4)\ \sqrt{(2lb + 2bt + 2lt)/6};\quad (5)\ \sqrt[3]{lbt}$$

(1) and (2) are arithmetical or statistical averages, (3) relates to the side exposed to view, (4) to the total surface, and (5) to the volume. In the calculation of the average size of the total number of grains, the value depends upon the weight given the factors of number, surface, and volume.

The various formulas may be written as follows:

1. Arithmetical or statistical mean diameter = $\Sigma nd/\Sigma n$
2. Length mean diameter = $\Sigma nd^2/\Sigma nd$
3. Average volume diameter = the cube root of $\Sigma nd^3/\Sigma n$
4. Surface average particle diameter = $\Sigma nd^3/\Sigma nd^2$
5. Weighted average particle diameter = $\Sigma nd^4/\Sigma nd^3$

Average particle size, as calculated by any of the above methods, does not indicate the uniformity of the material. This may be expressed fairly well by the "uniformity coefficient," $\sqrt{n/2\Sigma\delta^2}$, where δ is the variation of each size group from the average.

"Specific surface" is a numerical measure of the surface, in square meters, presented by one gram of the material. It is equal to $\frac{6}{\text{density x av. size}}$. Assuming the particles to be spheres, it can be shown that $psd = 6$, where p = density of material, s = specific surface (square meters per gram of material), and d = diameter of particle in micrometers. If the density is expressed in grams per milliliter and the diameter in μm., the specific surface will be in square meters per gram.

The "average size" used should be based on surface, unless the material is highly uniform.

Green's Method of Determining Particle Size

The number of particles of each different size is counted, tabulated, and plotted as a "size frequency curve." From the tabulation or the curve, the "numerical average particle size" and the uniformity coefficient may be estimated by inspection or may be computed.

Dry mounted preparations are preferable on account of their greater visibility and the absence of Brownian movements, and because the particles, if fairly uniform, are all in focus at the same time. The actual measurements may be made directly by means of an eyepiece micrometer or, preferably, by a photographic method. A total of 200 or more particles, occupying one or two representative fields, should be measured; if the distribution is uneven, or the uniformity is low, more measurements may be advisable.

Green's method is appropriately applied to material which is of microscopic dimensions, of good visibility, and relatively uniform in particle size, as exemplified by precipitated or "fumed" pigments or fillers (Table XXIII).

In Table XXIII δ is the deviation from the average particle size in micrometers. The values in the first column (mm.) are multiplied by 20,000, which is the magnification used for this sample.

The character and behavior of fine pigments depend to a large extent on the magnitude of their specific surface. Specific surface is inversely proportional to the diameter of the particle or particle size.

PREPARATION OF SLIDES OF PIGMENTS AND INERTS*

THE MULTIPLE WEDGE TURPENTINE DISPERSION (46)

The most useful pigment dispersions are those which are built up like wedges – that is, the particle density (number of particles per unit area of volume) varies gradually from a thin wedge to a thick one, as shown diagrammatically in Figure 74A.

On such a wedge mount, any degree of density can be found for inspection. At the thin edge of the wedge, perfect deflocculation usually exists, and such a spot can be used for photomicrography and particle measurements if desired.

The turpentine dispersion is made in the following manner: The pigment is rubbed with redistilled turpentine until all visible lumps are broken up. The rod is held firmly so that it does not twist in the hand, and, with a rotary motion in the plane of the slide, passed from one end of the slide to the other and backward again, following the path given by the arrow in Figure 74B. This process will distribute the pigments as shown in Figure 74A. The rubbing is continued until the turpentine is almost, but not quite, evaporated. (If the rubbing is continued too long, bunching and streaking of the pigment occurs). The slide is placed on the hot plate to drive off the last traces of turpentine. The mount is then ready for microchemical tests.

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*The preparation of the samples is the most important and the most time-consuming phase. Loveland (69) has an excellent discussion of sample preparation for particle size determination.

TABLE XXIII

PARTICLE SIZE DETERMINATION USING ZINC OXIDE (47)

mm.	f	f x mm.	δ	δ^2	$f\delta^2$
1	0				
2	0				
3	0				
4	0				
5	0				
6	8	48	4.44	19.71	157.68
7	6	42	3.44	11.83	70.98
8	19	152	2.44	5.95	113.05
9	53	477	1.44	2.07	109.71
10	82	820	0.44	0.19	15.58
11	46	506	0.56	0.31	14.26
12	34	408	1.56	2.43	82.62
13	12	156	2.56	6.55	78.60
14	5	70	3.56	12.67	63.35
15	2	30	4.56	20.79	41.58
16	2	32	5.56	30.91	61.82*
17	1	17	6.56	43.03	43.03
18	1	18	7.56	57.15	57.15
19	1	19	8.56	73.27	73.27
20	1	20	9.56	91.39	91.39
21	1	21	10.56	112.40	112.40
22	1	22	11.56	134.60	134.60
23	1	23	12.56	158.80	158.80
	276	2881			1479.87*

Magnification, 20,000 diameters

Average particle size = 2881/276 ÷ 20,000 = 0.522 μm.

Probable error = $0.6745 \sqrt{1479.87/(276 \times 275)} = \pm 0.094$

Coefficient of uniformity, $U = \sqrt{276/(2 \times 1479.87)} = 0.305$

Density = 5.78 grams per milliliter

Specific surface, $S = 6/(5.78 \times 0.522) = 1.99$ square meters per gram

*In the original paper this was given as 30.90 and the total as 1449. If the f and f x mm. columns are correct this must be in error.

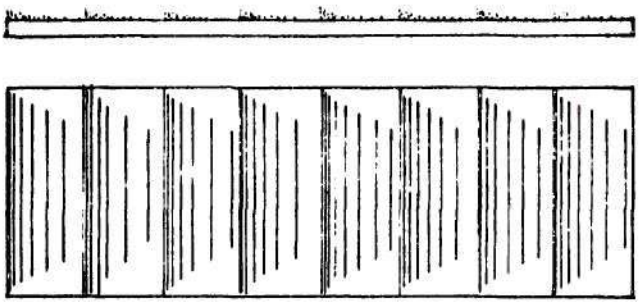

Figure 74A. Section of Plane of Turpentine Mount

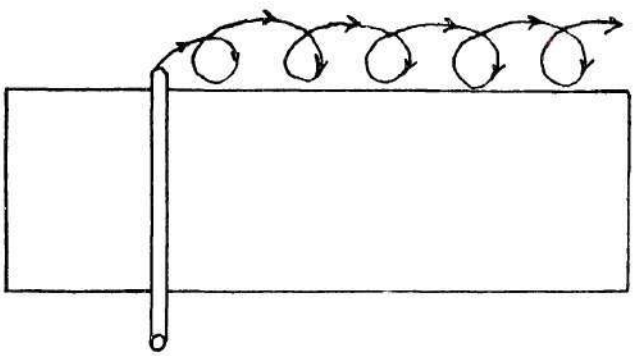

Figure 74B. Path Followed by Dispersion Rod

THE CIRCULAR WEDGE DAMMAR DISPERSION

The pigment is rubbed on the slide with turpentine until all lumps visible to the eye have been broken up, and the slide is dried on a hot plate. With a clean piece of linen all the pigment is removed except a small square, as shown in Figure 75A. The proper size of this square will depend on its density and is easily determined with a little practice. The more pigment it contains, the smaller should be the square.

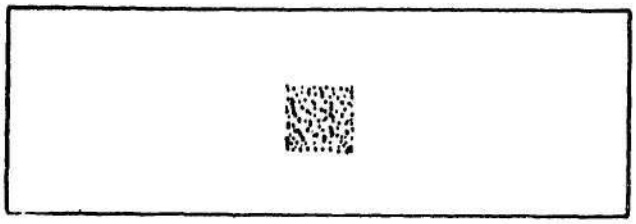

Figure 75A. Small Square of Material for Gum Dammar Mount

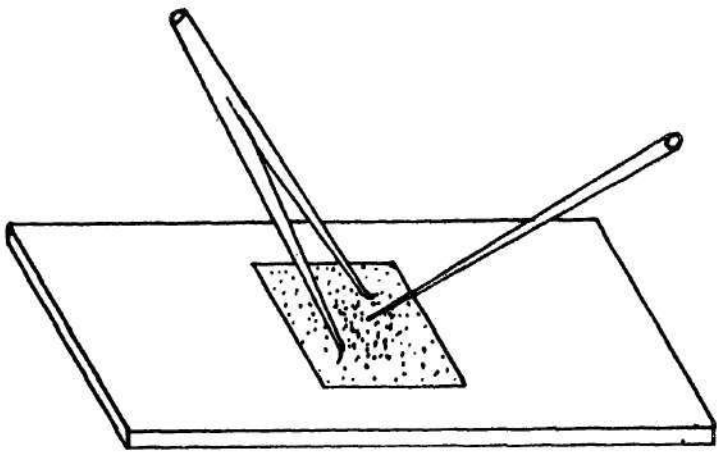

Figure 75B. Method of Dammar Mount Dispersion

A piece of dammar (purified by dissolving in chloroform, filtering, completely evaporating the solvent, and breaking into 5-mg. pieces) is placed on the square and melted on a hot plate. The cover glass is pressed down firmly to exclude excess dammar and bubbles. The slide is transferred to a cool table top on which has been placed a blotter (a dark one for white pigments). The cover glass is held firmly in position by pressing on it with the back of a spread pair of curved pointed forceps (Figure 75B). By means of a blunt stick the cover glass is pressed down at a central spot until all the pigment seems to be squeezed out from under the spot. During this operation keep the point of the stick tracing a small circle on the cover glass. This motion prevents flocculation. This is continued, always with

pressure, until the dammar solidifies. This mount will be suitable at the thin edge of the wedge for photography and particle measurements. It is of little service except at this thin edge, because that is the only spot where deflocculation is complete and the particles are approximately in one plane. If they are not in a single plane, the cover glass was not pressed down far enough.

The dammar mount, just described, is not used for microchemical tests; its purpose is to enable the investigator to make a careful inspection of the pigment by microscopical examination only. Having done this, it is often possible to tell just what pigments are present without proceeding further.

Turpentine and dammar have no power in themselves to deflocculate and hence to disperse. Dispersion is purely a matter of expert mechanical manipulation, and consequently requires practice. The dammar dispersions are the easier to make, but somewhat unsatisfactory in the hands of the novice, because grinding of the pigment may result from lack of sufficient experience. The beginner should master the turpentine dispersion before he attempts to go further.

A microchemical analysis of pigments does not require the same perfect dispersion as does a particle measurement by the photomicrographic method. Perfect deflocculation, in this case, is not essential for satisfactory results.

THE DUCO HOUSEHOLD CEMENT DISPERSION (48)

The pigment is dispersed in a high concentration of a suitable optically clear cement, thereby utilizing the high shearing forces which may be created in a stiff, viscous mixture (69). A satisfactory combination of the desired qualities is Duco household cement.

On a microslide is placed a drop of this cement and beside it a suitable quantity of the dry pigment. The pigment is kneaded into the cement with sweeping rapid strokes with a small spearhead dissecting needle, the cement hardening gradually as this operation proceeds. The strokes are 2- to 3-cm. long. After 8 to 10 strokes the mixture is rapidly scraped to a central point, from which it is spread out again immediately by additional rapid, swiping strokes. This operation of gathering and respreading should be repeated two or three times. Finally, the mass is gathered again and then spread quickly into a thin layer. The entire rubbing operation consumes 20 to 40 seconds.

The mix at this state is too concentrated for microscopic examination and must be diluted. For this purpose a very small piece (about 0.5-mm. square) of the hardened mix is cut off by the point of the spearhead, placed on a clean slide, and covered with a small drop (2 mm. in diameter) of fresh Duco cement. This combination is then rubbed out gently under a cover glass by imparting a squeezing and rotating motion with the index finger, which is protected with a thin, clean cloth to prevent soiling the cover glass.

In this method the high viscosity of the matrix, even in fresh preparations, prevents movements of the individual particles sufficient to be annoying for observation; if however, the preparation is to be photographed with an exposure of many seconds duration, it should be allowed to stand for a half hour or more before the particles are sufficiently motionless.

THE AMYL ACETATE PARLODION DISPERSION (49)

The dispersion medium first developed and most extensively used consists of the following formula: 82 g. amyl acetate, 6 g. ethyl ether, 4 g. absolute ethyl alcohol, and 1.94 g. parlodion.

In use, this formula evaporates rapidly and is satisfactory for most powders whose individual particles are dense and of considerable structural strength. For powders in which the particles are fragile and which must be handled more cautiously, a solvent mixture which evaporates more slowly appears to possess some advantages. A modification of the above formula is: 80 g. amyl acetate, 4 g. ethyl ether, 6 g. absolute ethyl alcohol, 2 g. butyl alcohol, and 1.94 g. parlodion.

Procedure

The parlodion in flake form dissolves slowly in the mixed solvents. The method used is: Place a little of the powder on a clean glass slide. Put a drop or two of the parlodion solution over the powder and disperse by rubbing with a small spatula. Let one drop of the dispersion fall on the surface of a beaker of water and stand for a minute or two until the solvent sets, changing from shiny to dull. Cut away part of the film and remove it with a clean spatula. Slip a clean glass slide under the remaining film so that it touches the film under water in a horizontal position. Raise the slide slightly above the surface of the water and cut away the film along the edge of the slide. Touch the edge of the slide to absorbing paper to draw off the water. Air dry. Examine under the microscope or add a drop of Clarite and cover with a cover glass.

ELIMINATION OF PIGMENTS FROM A MOUNT

It is sometimes desirable to eliminate one or two pigments from a dispersion so that the remaining ones can be tested properly. Elimination is carried out by the use of solvents; they are always made on turpentine dispersions.

When a turpentine dispersion is made correctly, the pigment particles adhere to the slide with sufficient tenacity to permit washing with solvents without loosening the pigments which are unaffected by such solvents. To make an elimination, the dispersion end is placed into a beaker containing the solvent. The slide is allowed to remain against the side of the beaker for the

required time, and is then rinsed in another beaker containing distilled water.

A half elimination is one in which the dispersion is immersed to only one-half of its depth, using only half as much solvent as for the full elimination. A half elimination gives two fields on the same slide, one attacked and the other unattacked by the solvent. This form is very useful for a macroscopic as well as for a microscopic test.

PHOTOGRAPHIC METHOD FOR DETERMINING PARTICLE SIZE

PREPARATION OF THE SAMPLE

The ordinary turpentine suspension is to be used principally with the finest pigments, such as zinc oxide, lithopone, white lead, etc. With coarser material, such as clays, barytes, asbestine, etc. (which will probably feel gritty), only the slightest possible pressure must be brought to bear upon them with the dispersing rod, for there is danger of a real grinding effect during the dispersion. Fortunately, these materials are so large that flocculation does not prevent the outline of the particles from being seen and measured; hence, very little rubbing is necessary.

Upon microscopic observation three essential conditions should be manifest: 1. The particles should be in one plane. 2. They should be free from Brownian movements. 3. They should be dispersed, showing individual grains instead of aggregates and flocculates.

PHOTOGRAPHY

All photographs are made with transmitted light, and absolutely axial illumination. Oblique illumination will give a distorted image, causing an appreciable error in the results.

MAGNIFICATION

As structure is of minor importance, magnifications which are too large for definition can be used, as long as the edges of the particles are sharply defined. With a 2-mm. apochromatic objective a magnification of 1500 diameters will be most convenient.

MEASUREMENTS

The negative, which must show from 200 to 250 distinct particles, is placed in a stereopticon and an image is thrown on a screen, so situated that

the total magnification of the original particle will be from 20,000 to 25,000 diameters. The largest diameters of the particles are measured in one direction only – the horizontal direction.

AVERAGE DIAMETER

If the average particle volume is desired, it is necessary to base the calculations on some assumption as to the shape of the particles. It is usually customary to assume that they are spherical in form. It should be noted, however, that by this method of particle measurement it is not necessary to fall into the error of using the cubed average diameter in place of the average of the cubed diameters. In order to avoid this mistake it is only necessary to replace the column $\underline{f}$ x mm. with $\underline{f}$ x $mm.^3$.

ERRORS AND MISTAKES

I. Errors of Magnification

1. Calibration of stage micrometer
2. Measurement of image of micrometer on glass focusing screen
3. Measurement of bellows length
4. Determination of the stereopticon magnification: $\underline{a}$ – the ruled lines; $\underline{b}$ – image on screen
5. Distortion of image on focusing screen

II. Errors in Obtaining Average Diameter of Particle Aside from Errors in Magnification

1. Errors due to the fact that the number of measurements cannot be infinitely great
2. Errors from disregarding fractional parts of millimeters in measureing the images of particles
3. Errors arising from the fact that the largest particles do not always occur with the proper frequency on a single negative showing 200 to 300 particles
4. Errors from poor judgment in selecting a representative section for photographing
5. Errors from diffraction defects when particles are photographed, the diameters of which are less than the resolving power of the objective
6. Errors due to distortion of the photographic emulsion upon drying

III. Negligible Errors

1. Errors in ruling of the millimeter scale for the determination of the bellow length
2. Errors in the ruling of the scale used in measuring the image of the stage micrometer
3. Errors in the comparator

IV. Mistakes

1. Poor focusing
2. Oblique illumination
3. Insufficient and excessive illumination
4. Underdevelopment and overdevelopment of negatives

HEMACYTOMETER CHAMBER METHOD (50, 51)

The following procedure, which has been used for a long time in medicine and physiology, but which, in spite of its numerous practical applications, has found little or no use in industry, is based on the quantitative counting of finely divided products by means of the hemacytometer chamber commonly used for counting blood corpuscles.

By means of this method, the important pigments used in paints, printing inks, and wallpaper colors can be investigated. If one desires to follow the progress of the dispersion or crystallization of a finely divided material, microscopic methods are often impossible. The subdivision, as well as the increase in the size of the crystals, does not occur uniformly in all parts. The proportions of differently sized particles vary for each test and, therefore, an answer to questions of this kind can be obtained most easily by the quantitative determination of the fineness of the average of the finely divided substances, for which the hemacytometer is well suited.

The standard hemacytometer consists of a cell 0.1-mm. deep and 1-mm. square. It is ruled into tiny squares of 1/400 square millimeter areas. There are two such cells on the hemacytometer.

A quantity of the material for size determination is weighed on a chemical balance and transferred to a graduate. A viscous liquid is added until a definite volume of solid plus liquid is obtained. The weight and volume is subject to individual preference, but 0.1 to 0.2 g. of ground material with enough liquid to make 2 ml. is ordinarily satisfactory for 200-mesh material. The liquid should be viscous to prevent rapid settling of the particles after being stirred and should be soluble so that it can be washed off by placing the cell under the tap. Glycerin or Karo syrup is satisfactory.

Thorough stirring can be readily accomplished by blowing into the suspension through a glass tube. When a uniform suspension has been produced, a drop is removed from the glass stirring tube, and the suspension is allowed to creep under the cover glass of the cell by capillarity. It is important that the sample be introduced in this fashion to avoid erroneous results. Placing a drop on the cell and then laying on the cover glass will usually lead to a higher than normal particle count.

The cell is then allowed to rest for about 30 minutes to permit all particles to settle to the bottom of the cell. This is not absolutely necessary, but it is convenient because it avoids having to change focus constantly during the counting. A 4-mm. objective is used, preferably one of the newer types with a large working range. A mechanical stage is also convenient but not necessary.

It is not necessary to count the total number of particles in the entire cell. Enough of the cell is mounted to give 500 to 600 particles and the volume of the suspension involved is determined by the number of the 1/400 sq. mm. areas counted. It is well to count a portion of the total from each of the two cells of the hemacytometer. The number of particles in a known volume of suspension is thus determined, the number of particles in the original weight of the material taken can then be calculated, and the average weight per particle secured. The volume of a particle of average weight can be secured by dividing the weight by the specific gravity of the material and then calculating the diameter.

Assuming the particle to be spherical, the above operations can be combined in the formula:

$$d = \sqrt[3]{6wV_2/\pi GV_1 n}$$

where d = average diameter (cm.), w = original weight of solid (g.), V_2 = volume in milliliters of the suspension counted, G = specific gravity of the solid, V_1 = total volume of suspension, and n = number of particles counted.

The possibilities for employing the hemacytometer for counting the particle size of precipitates and for the criticism of the different precipitation methods should be emphasized. Among the uses are the study of the effect of dilution, the influence of temperature and, therefore, the reaction velocity, the effect of standing (the precipitate will become denser upon long standing and, therefore, can be filtered more easily), the effect of acid, alkaline, and neutral solutions on the solubility of a material, and also the action of protective colloids on the precipitation.

The accurate estimation of grain fineness is of value, not only for colors, but for other technical products as well; this is true, especially, in cases where the higher degrees of grain fineness are of use; where, because of it, intimate mixing is produced and, thereby, insoluble bodies react more easily with each other. It is also important where absorptive capacity is increased by a higher degree of fineness, or where the fineness of the grain determines the luster and hardness; this is the case with glossy papers, cements, polishing materials, abrasives, and many other products.

AUTOMATIC AND SEMIAUTOMATIC SCANNING DEVICES

ZEISS PARTICLE SIZE ANALYZER (after Endter)

The analyzer is a semiautomatic device in which the eye and judgment of the operator participate in the measuring process, which is done from enlarged micrographs. The apparatus consists of a light source, an iris diaphragm, and a condensing and projecting lens system. The iris diaphragm is adjustable, and the different diameters of the iris are correlated, via a commutator, with 48 counters, each of which covers a certain range of iris diameters. A counter registers the total number of particles measured; distribution of particle diameters can be recorded arithmetically, exponentially, or cumulatively (61, 70).

FLYING SPOT MICROSCOPE

The method that uses scanning of the microscopic field by a small spot of light produced by the combination of a standard microscope and a conventional television flying spot scanner cathode ray tube has proved to be both practicable and versatile. The specimen is scanned by a spot much smaller than the particles in the field. As the spot crosses opaque areas in the specimen, pulses are generated and passed on to an amplifier whose output controls a display tube. For counting and measuring, the preparation is scanned not by one but by two spots, one line width apart, recording on separate photocells. To simplify equipment the maximum dimension of the particle in the direction of scan is taken as the measure of the size of the particle, the error being subsequently reduced by taking successive scans with different orientations of the field. In this method a pulse of known duration is generated by the leading edge of each intercept. For particles whose maximum intercept is less than the pulse duration no count is registered. Successive scans are made with various pulse durations and a cumulative distribution curve is obtained (61, 71).

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CHAPTER VI

FIBER MORPHOLOGY

Although wood is by far the most widely used raw material for paper pulp, many other vegetable fibers as well as animal, mineral, regenerated and synthetic fibers are utilized. All these fibers may enter the mill in the form of virgin or waste material.

Certain of the fibers are readily identified but often the separations are difficult, or even impossible. Difficult identifications may be due to inherent similarities in morphological characteristics or to the mechanical and chemical actions to which the fibers are exposed during the pulping, bleaching, refining, and papermaking processes, which may obscure or obliterate the details requisite for identification.

While it is our intent to orient the reader in the morphology of wood and other fibers in this chapter, it will be desirable for him to refer to additional sources for further detail. A basic knowledge of plant anatomy, especially wood anatomy, and a familiarity with the fibers used in the textile and cordage industries is of distinct value to the pulp and paper microscopist (1-3, 46). It is also important that the analyst be familiar with the man-made fibers as some of them are met with in the industry (3, 47).

The analyst will find it profitable to maintain a file of authentic specimens of the fibers he is apt to require in his particular situation.* For most mill analysts this will be a limited number, the size of the group naturally varying according to the types of pulp and paper manufactured.

Although in reality there is no good substitute for experience with authentic specimens, the analyst will find it worthwhile to have drawings, photomicrographs, and keys at hand to assist him in his identifications. These devices are particularly helpful if new fibers are encountered from time to time.

CLASSIFICATION OF FIBERS

Before proceeding with a detailed description of the morphology of the various fibers used in the paper industry it seems desirable to outline a general classification of fibers. Several systems have been offered by various authors during recent decades. These systems, like that of the writer which is presented below, have their advantages and disadvantages.

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*The TAPPI Fibrary, of which The Institute of Paper Chemistry is custodian, has several hundred authentic samples. The catalog of fibers and the directions for ordering them may be obtained from the Institute.

It may be appropriate, in review, to list the principal factors which determine whether a fiber will be used in the manufacture of paper or paperboard. These are suitability of fiber, dependability of supply, cost of collection, transportation, preparation, and resistance to deterioration.

FIBER CLASSIFICATION

I. Plant Fibers

- A. Fruit fibers
  - 1. Seed hairs-cotton, milkweed
  - 2. Pod fibers-kapok
  - 3. Hull fibers-coir
- B. Stem fibers
  - 1. Wood fibers-gymnosperm and angiosperm
  - 2. Bast fibers
    - a. Trees and shrubs-gymnosperm and angiosperm
    - b. Herbaceous dicotyledons-flax, hemp, sunn, jute, ramie
  - 3. Vascular bundles of monocotyledons-cereal straws, bamboo, bagasse, cornstalks, esparto
- C. Leaf fibers-abaca, sisal, henequen, pita, phormium, pineapple, caroa, Mauritius hemp, coconut palm

II. Animal Fibers

- A. Silk
  - 1. Cultured silk
  - 2. Wild silk
- B. Wool
- C. Specialty hairs

III. Mineral Fibers

- A. Asbestos
  - 1. Chrysotile (magnesium silicate)
  - 2. Crocidolite (iron silicate)

IV. Man-Made or Artificial Fibers

- A. Glass fibers
- B. Regenerated cellulose fibers

1. Viscose rayon
2. Cuprammonium rayon
3. Fortisan (regenerated acetate rayon)

C. Cellulose ester fibers

1. Acetate rayon

a. Secondary acetate
b. Triacetate

D. Protein-base fibers

1. Casein fibers
2. Soybean fibers
3. Peanut fibers
4. Zein fibers

E. Carbohydrate fibers

F. Polyamide fibers

G. Polyvinyl fibers

H. Polyacrylic fibers

I. Polyester fibers

J. Polyethylene fibers

MORPHOLOGICAL CHARACTERISTICS

WOOD FIBERS

The spermatophytes, or seed-bearing plants, which comprise the largest of the divisions of the plant kingdom are divided into two groups known as the gymnosperms and the angiosperms. The only order of commercial importance in the gymnosperms is the coniferales, commonly called the conifers, or cone-bearers. The conifers generally have needlelike leaves and most of the species are evergreen but there are some which shed their leaves at the end of the growing season (deciduous). Foresters, lumbermen, wood technologists, and others commonly refer to the coniferous woods as softwoods.

The angiosperms, which have covered seeds in contrast to the naked seeds of the gymnosperms, are subdivided into two classes – the monocotyledons and the dicotyledons. The monocotyledons are characterized by one seed leaf (cotyledon), flower parts in threes, and vascular bundles scattered through the ground tissue of the stem. The dicotyledons in turn have two seed leaves, flower parts in fours or fives, and a stem with one solid, or perhaps dissected, vascular bundle. The trees of the dicotyledons are usually broadleaved. Many of them have deciduous leaves but others have evergreen leaves, particularly the species in the warmer climates. Foresters, lumbermen, wood technologists,

and others usually refer to the dicotyledonous woods as hardwoods. It must be emphasized that the terminology, softwood and hardwood, does not refer to the physical hardness of a particular species as some softwoods are harder than some hardwoods.

ANATOMY OF THE CONIFEROUS WOODS

The coniferous woods, or softwoods, are much simpler anatomically than are the hardwoods (1). The cell types which may be present in a coniferous wood are tracheids, longitudinal wood parenchyma, ray tracheids, ray parenchyma, and epithelial parenchyma. This is about half as many cell types as are present in the hardwoods.

The cell element of greatest importance to the papermaker, fortunately present in a volume exceeding 90% of the wood, is the tracheid, or "fiber" as it is known in the parlance of the industry. The tracheid has a length 50 to 100 times greater than its diameter. The average length for unbroken softwood tracheids is 3 to 4 mm., with a normal distribution for a species and variation among species. The radial walls in particular contain a large number of openings in the secondary wall called pits (Figure 76). The larger doughnut-shaped pits scattered the length of the radial wall of the tracheid (especially the springwood tracheid) are known as bordered pits. At irregular intervals in the length of the tracheid are pit areas which differ in appearance from the large bordered pits; these are cross-fields. The pits of the cross-fields tend to be influenced in size and shape by the complementary pit in the adjacent ray parenchyma or ray tracheid cell. In general, pits leading to the ray tracheids are quite small and bordered. The pits connecting to ray parenchyma cells, however, show considerable variation in several species and hence can serve for identification of certain species or groups of species (4,5). Examples of some of the principal types of cross-field pitting are illustrated in Figure 77.

The relatively short ray cells and the similar-appearing longitudinal parenchyma cells and epithelial cells constitute the fines along with slivers and small pieces broken off from the fibers. In chemical pulps they are of no diagnostic value but in wood and often in mechanical pulps their arrangements and presence or absence can be important in identification. The references previously mentioned in this section will be a valuable aid in the species identification of the softwood fibers.

ANATOMY OF THE DICOTYLEDONOUS WOODS

The trees among the dicotyledonous plants grow wood or xylem which is considerably different than coniferous wood in its structural detail (1). These woods, commonly called hardwoods, are usually more complex in their structure than the softwoods. The characteristic feature of the hardwood is the vessel (known as the pore in cross-sectional view). The use of the term pore in this sense is responsible for these woods being known collectively as porous woods. This contrasts with the term nonporous woods used to designate the coniferous woods which do not contain vessels.

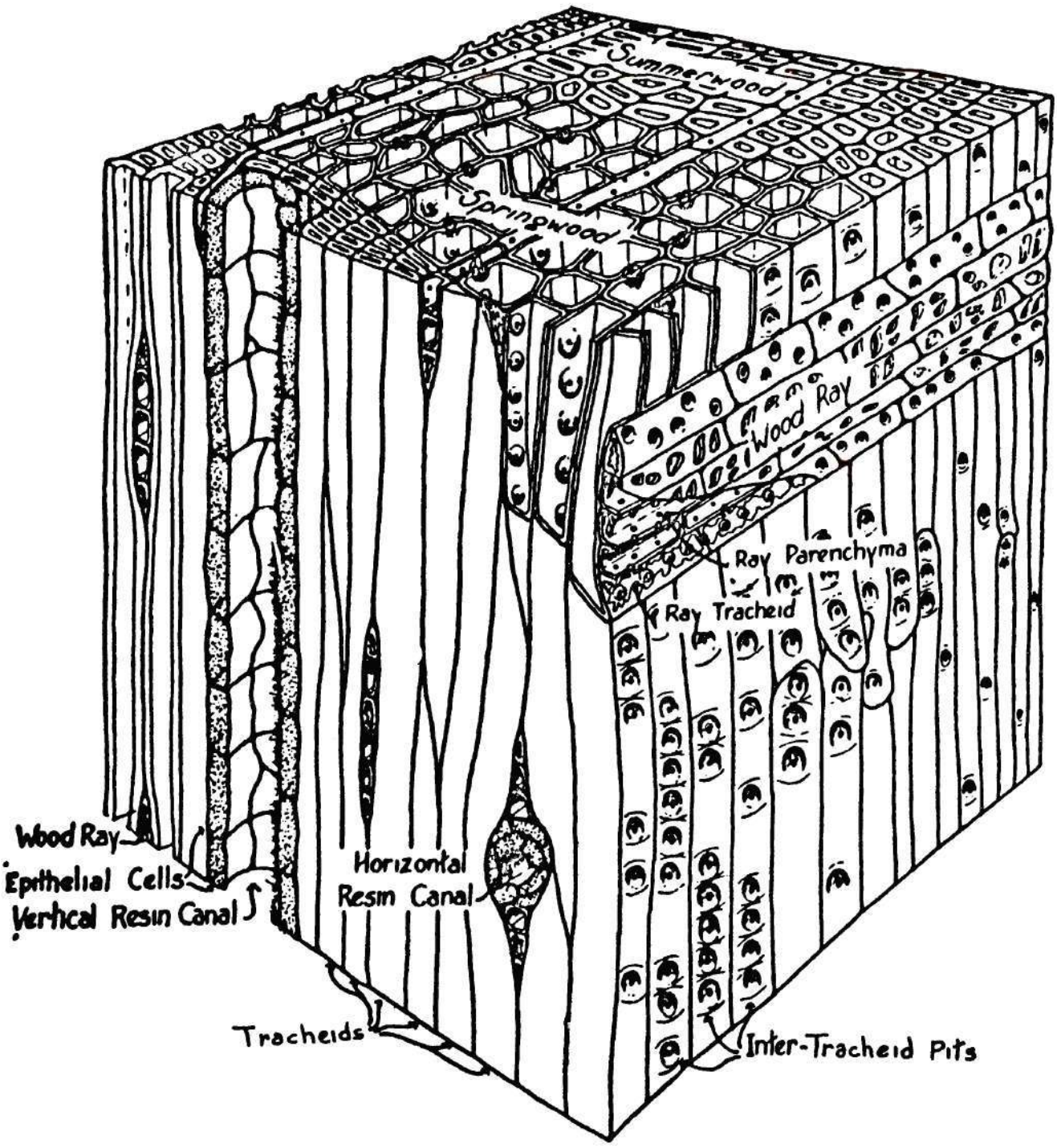
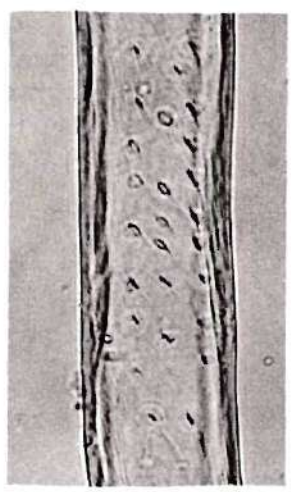


Figure 76. Hard Pinewood (Adapted from Forest Products Laboratory)

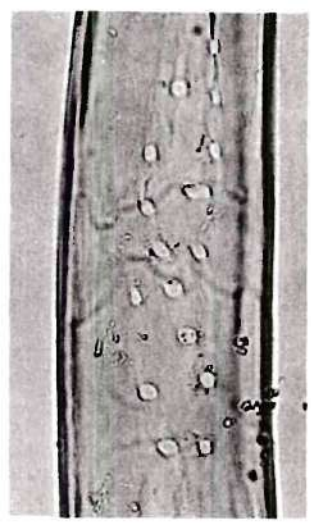
A. White Pine
X200

B. White Pine
X200

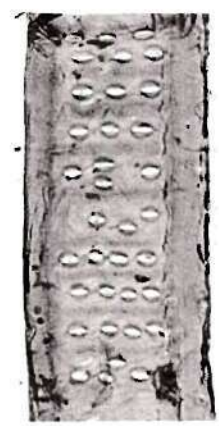
C. Slash Pine
X200



D. White Spruce
X400



E. Balsam Fir
X400



F. Redwood
X200

Figure 77. Cross-Fields in Softwood Tracheids

The vessel runs longitudinally for an indefinite length in the tree and is composed of countless members or segments connected end to end much as tile sewer pipe or stove pipe (Figure 78). When the wood is chemically pulped the various segments are usually present in the pulp although they may be broken. The vessel members of different species are often quite distinctive and are very helpful in the identification of hardwood pulp species (5, 48). Isolated vessel members from a few American species are shown in Figure 79 to illustrate the general appearance and possible variations but the references (5) should be consulted for additional information. Conspicuous features on the vessel member of value in identification are the perforations at or near the ends of the cell, presence or absence of spiral thickening, type, size and arrangement of pits leading to adjacent vessels, rays, fibers, tracheids, and longitudinal parenchyma (5). The perforation is either simple, i.e., an unobstructed hole (Figure 80A), or scalariform, showing ladderlike bars across the opening (Figure 80B).

The most important cell types in hardwoods from the papermaking standpoint are the libriform fiber and the fiber tracheid. These cells resemble the softwood tracheid in general shape but are usually shorter, narrower, and considerably less pitted. With occasional exceptions because of spiral thickening, wall thickness, fiber length, or the like, they are of little aid in identification of species in pulp form. The presence of the cell type known as the tracheid – a shorter, irregular-shaped cell with large bordered pits – is valuable in indicating the presence of one of only a few possibilities, such as oak or chestnut (Figure 81). Other cell types found in hardwood pulps are parenchymatous in nature and may be from the rays or the longitudinal strands of parenchyma cells. These are short cells with relatively thin walls and simple pits. Certain species, mainly tropical, show epithelial parenchyma surrounding gum canals.

NONWOODY VEGETABLE FIBERS

The nonwoody vegetable fibers are derived from several sources in the plant: seed hairs, fruit husks, bast fibers, stem vascular bundles, and leaf vascular bundles. At the end of the chapter are listed several references describing these various classes of papermaking fibers (3, 5-10, 49), but for most purposes TAPPI method T 10 m-47 includes the fibers other than wood which are normally encountered in the industry. For convenience many of these fibers are discussed in the following sections of this chapter.

Many of these fibers are also used for textile and cordage purposes. For this reason the American Society for Testing Materials (11), the American Association of Textile Chemists and Colorists (12), and the Textile Institute (13) have methods which are suitable for the identification of many of these fibers before they have been processed and altered significantly chemically. These methods are frequently valuable aids in the identification of these fibers even after pulping, bleaching, and refining, but they must be used with caution.

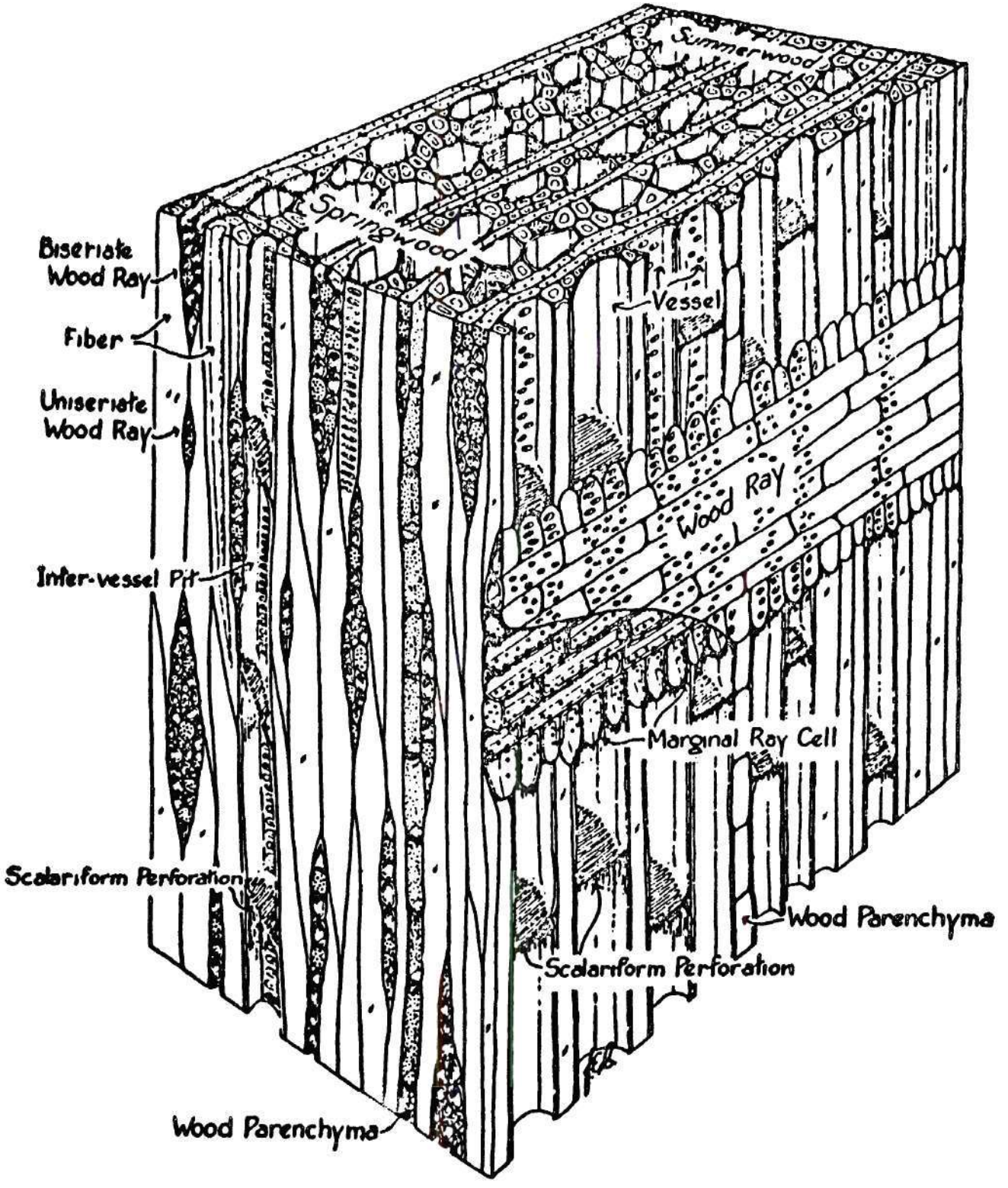
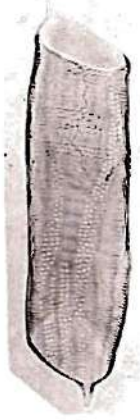


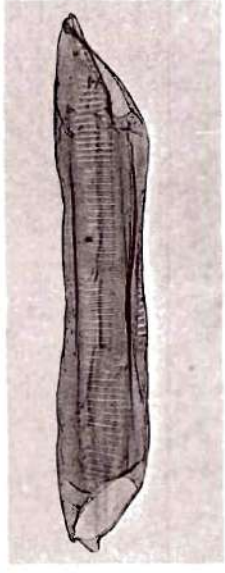
Figure 78. Gumwood (Adapted from Forest Products Laboratory)

A. Buckeye
X 100



B. Sugar Maple
X 100

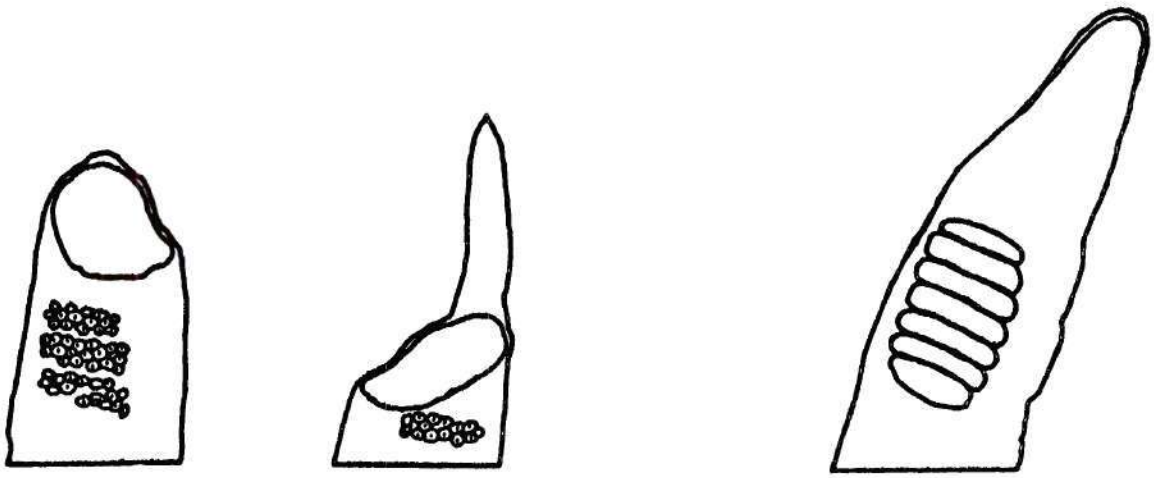
C. Basswood
X 100



D. Cucumber Magnolia
X 100

E. Red Oak
X 100

Figure 79. Hardwood Vessel Members



A (Simple) B (Scalariform)

Figure 80. Vessel Perforations X200

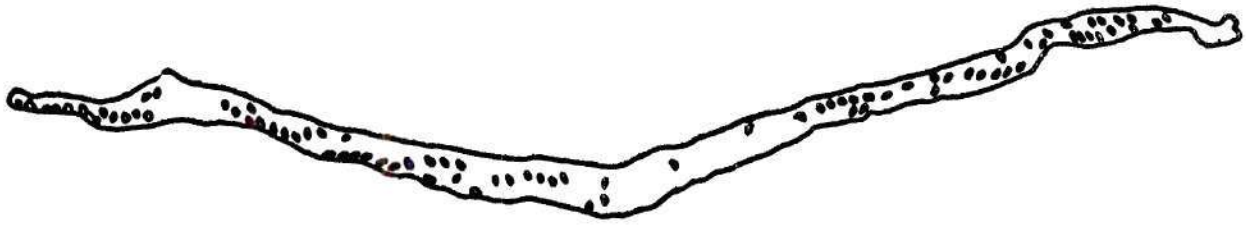


Figure 81. Oak Tracheid X200

Fruit Fibers

Cotton

The seed hair most commonly used in the paper industry is that of the cotton plant. There are several species and varieties of cotton, a discussion of which is beyond the scope of this text. The species of outstanding importance in the United States is American Upland (Gossypium hirsutum).

In the past most of the cotton fiber used in papermaking began as textile fiber and entered the paper mill as new cuttings or as old rag. The use of cotton linters, the short (2.0-5.0 mm.) fuzzy fibers firmly attached to the seed, has increased greatly in recent years because of the short supply of acceptable rags.

The cotton fiber, as usually seen, has the appearance of a flat ribbon, more or less twisted on its longitudinal axis (Figure 82A). This is the mature seed hair, the walls of which have collapsed. The length, 12 to 33 mm., is much greater than the diameter, 16 to 22 μm. Close examination of the fiber ends would reveal one pointed, the tip of the cell, and the other torn, the base where it had been attached to the seed coat of the cotton seed. Immature fibers usually do not show the twist. In cross section the immature fibers show only a single line with no structure and but little or no indication of an internal opening. The mature fiber is thicker in cross section and exhibits a central cavity or lumen (Figure 82B).

Polarized light is very helpful in separating mature from immature cotton fibers (14). A polarizing microscope with a magnification of 100 diameters, is adjusted so that the nicol prisms are crossed, the eyepiece is set with the cross hairs at 45° to the plane of polarization, and a selenite plate (red, first order) is placed in position. Cotton fibers mounted on a slide parallel with respect to the arrow on the selenite plate, are then examined. Very immature fibers appear violet or indigo, immature fibers blue, mature fibers green or yellowish green, and overmature fibers yellow. When rotated through 90°, the violet fibers become orange, blue fibers change to yellow, yellow fibers become yellowish green, and yellowish green fibers do not alter. Immature fibers exhibit almost completely parallel extinction when rotated through 360°. The color changes following the rotation of the field serve to check doubtful appearances.

The microscopic structure of cotton linters is similar in many respects to that of staple (lint) cotton (15). There are no pronounced differences between these two types, although in general, linter fibers are darker, shorter, more nearly cylindrical, and have thicker walls and narrower central canals than the staple cotton. The thin primary wall, which covers the surface of the fibers, consists largely of waxy and pectic materials. The thick secondary wall is arranged in layers. In some linters this layered pattern is due to the alternation of layers of cellulose which differ in porosity and density, and in others to the alternation of layers of cellulose with layers of noncellulosic material. The width of the individual layers varies roughly between 0.1 and 0.4 μm. in unswollen fibers. The cellulosic layers are further subdivided into fine threadlike fibrils which make an acute angle with respect to the long axis of the fiber. The orientation of the

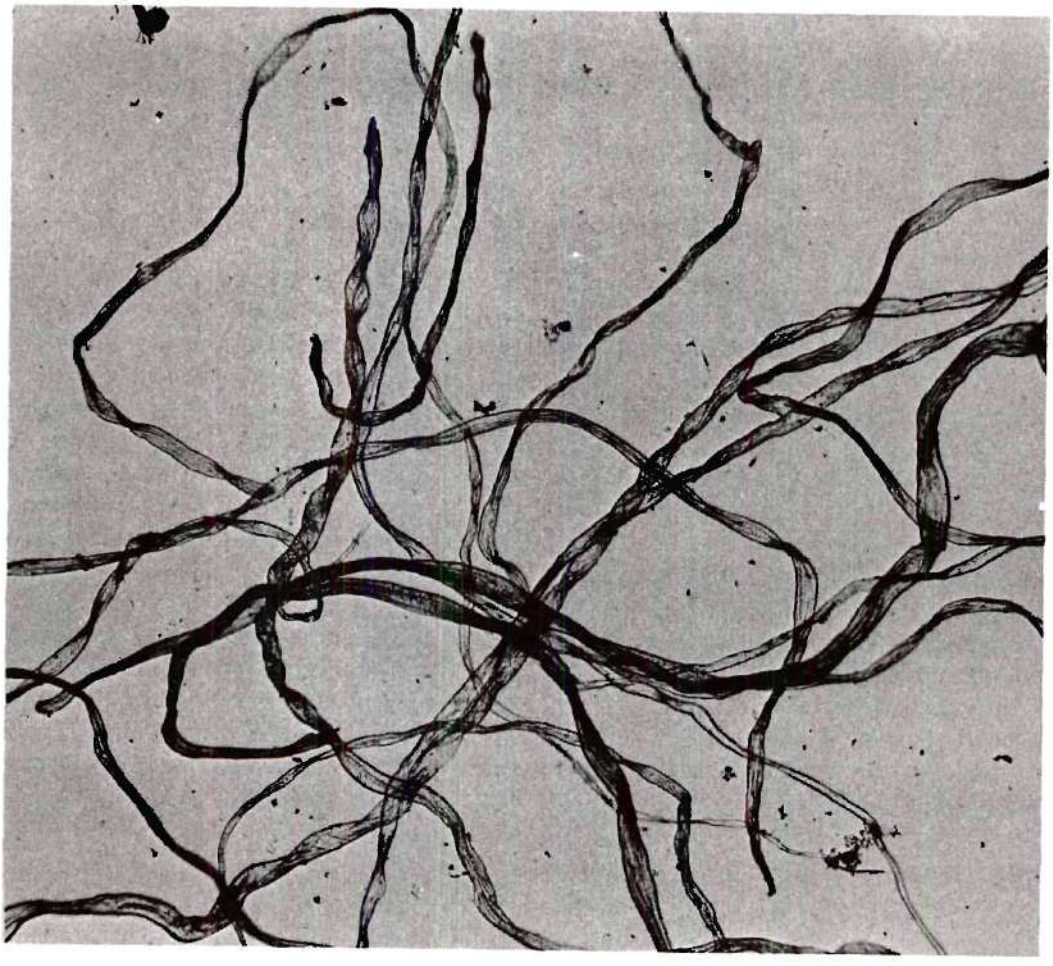


Figure 82A. Mature Cotton Fiber (X100)

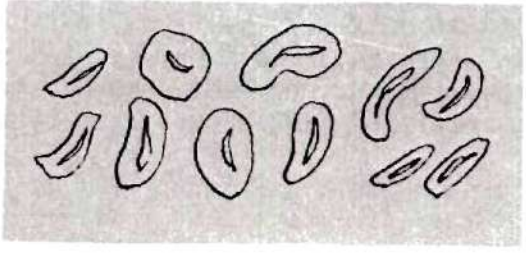


Figure 82B. Cross Section of Cotton Fiber (X330)

fibrils is not the same in all layers and may even reverse its direction of spiral in a single layer. These structural characteristics influence the manner of swelling.

Kapok

The kapok fiber is obtained from the pod of the kapok tree (Ceiba pentandra). The tree is indigenous to South America but most of the commercial production of the fiber is now centered in the island of Java.

The length of the unbroken kapok fiber ranges from 10 to 30 mm., with an average length of 18 mm.; the fiber width is 25 to 40 μm. The fibers are generally circular or oval in cross section with a very thin wall and a very large lumen (Figure 83). Unripe or dead fibers appear flattened in cross section. The fibers have a beautiful silky luster, are yellowish brown in color, and are very light in weight.

The fiber has a tapering cylindrical form, a bulbous base, and resembles a smooth, transparent, structural rod, frequently doubled over on itself. The resistance of the thin wall to natural conditions is fairly high, but it offers less resistance to the wear and tear of the working into yarn. It is soft and too inelastic for spinning; it is used in life preservers, mattress stuffings, and as filler (3).

Coir

Coir fiber is obtained from the husk of the fruit of the coconut palm (Cocos nucifera), from which it is separated and cleaned mechanically after the shell has been soaked for considerable time in sea water (16). Coir fiber has always been processed on an industrial scale in only two areas, Ceylon and the Malabar Coast of southwest India.

The long brownish fibers extracted by this retting process are actually vascular bundles composed chiefly of shorter fibrous elements (17). The ultimate fibers are 0.4 to 1.0 mm. long and usually 15 to 18 μm. wide although the width varies from 10 to 25 μm. (18); they are oval in cross section. The fiber-wall thickness is about one-third of the cell diameter in the majority of cases, but the thickness varies in different parts of individual fibers, so that the lumen is seen to vary in width when the fiber is examined longitudinally. The fibers have pointed, occasionally blunt ends and numerous minute pits will be seen in the walls. Diagonal striations will be observed between these pits.

Small siliceous objects, round in shape and lenticular in section, will be found adhering to the walls of the larger bundles of fibers. These are called stegmata, and will fuse together to form hard siliceous globules if fibers are ashed at 900°C. Further, if the fibers are boiled in nitric acid before being ashed the stegmata will then form "strings of beads" easily recognized under the microscope. Stegmata are usually 12 to 15 μm. in diameter. Individual fibers which were located at the outside of the bundle show these stegmata or else a waviness in the wall where the stegmata were located (3).

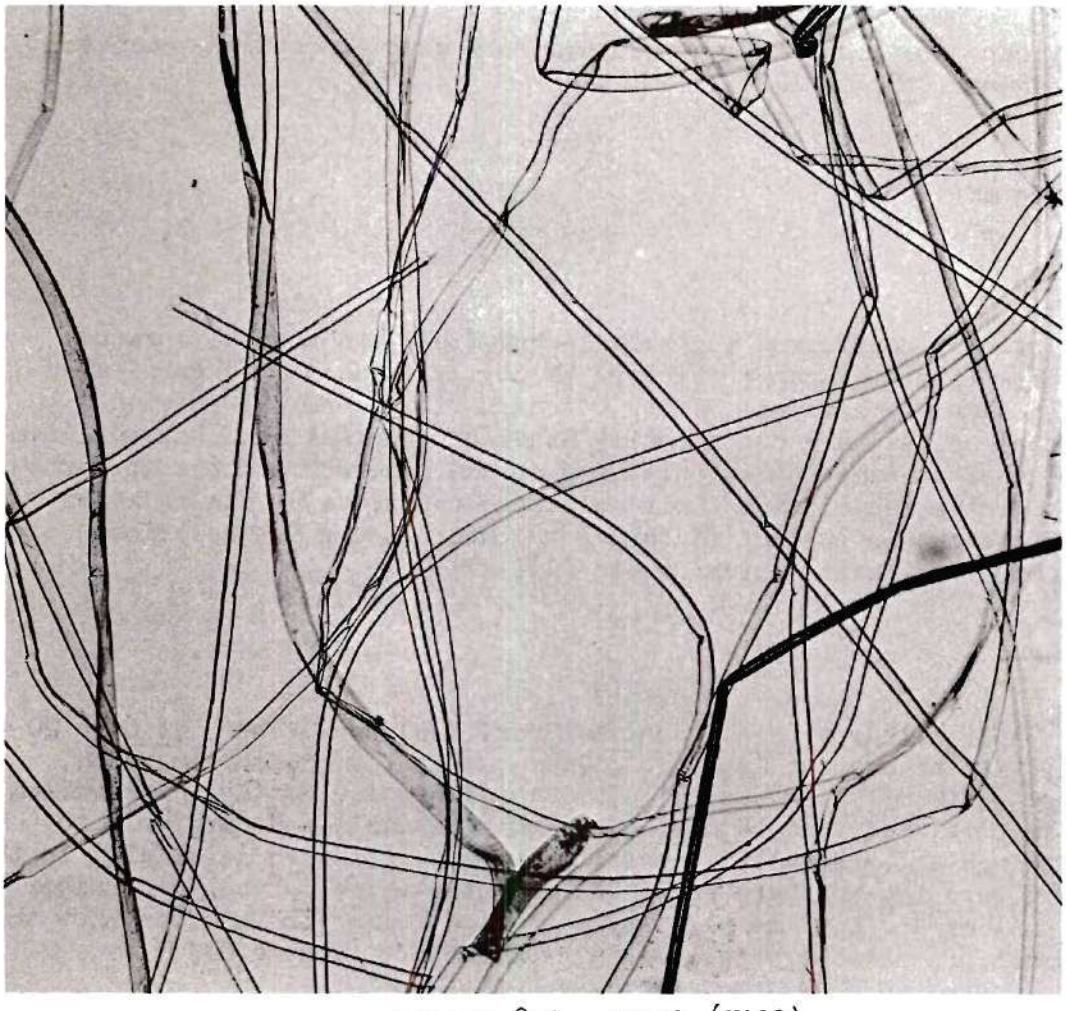


Figure 83A. Kapok (X100)

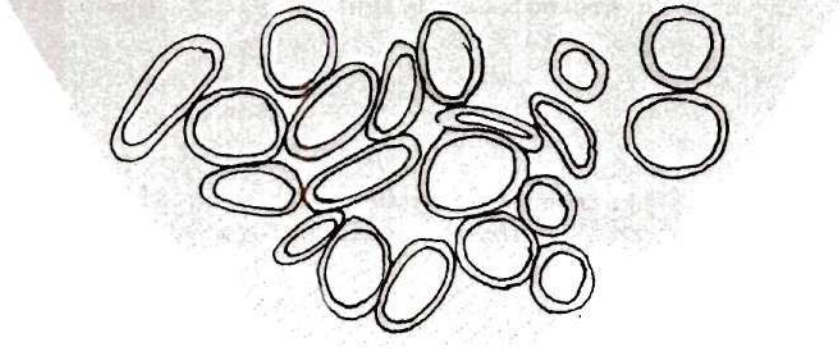


Figure 83B. Cross Section of Kapok Fiber (X380)

Coconut fibers are lignified and consequently give a positive reaction with lignin reagents (18).

Bast Fibers (19, 50)

Trees and Shrubs

The bark of many coniferous and broadleaved trees and shrubs contains fibers. The bark fibers of paper mulberry (kozo), mitsumata, and gampi are used in certain Japanese handmade papers but bark fibers and the related sclereids – stone cells – are not normally used for papermaking. However, the fact that bark does at times enter pulp, paper, and paperboard makes it worthwhile to acquaint the reader with their existence and to refer him to a few general references on the subject of bark structure. Chang has described the bark anatomy of various American trees (20, 21).

Kozo

Kozo, or paper mulberry (Broussonetia papyrifera) bark fiber, is 6 to 20 mm. in length, with an average length of 10 mm., and 25 to 35 μm. in width, with an average width of 30 μm. (6, 8, 9). The fibers are mostly thick-walled and are sometimes twisted like cotton. The lumen is small and difficult to distinguish, though at intervals it is filled with a yellowish material. In the ribbon-shaped fibers the ends are broad and rounded, while in the thick fibers the ends are smaller and tend to be sharply pointed. The fibers often show the presence of small prismatic crystals of calcium oxalate (Figure 84).

Mitsumata

Mitsumata (Edgeworthia papyrifera) fibers are of highly characteristic structure and usually are easily recognized. They are very irregular, ranging in width from 12 to 27 μm., with an average width of 18 μm. The fiber length is 2.4 to 3.6 mm., with an average length of 2.9 mm. The ends are mostly rounded, or broadened, less often pointed, and are often forked or branched; the lumen is often interrupted, the thickness of the walls varying greatly (Figure 85). The fibers are not lignified and yield the finest Japanese papers (7, 8, 9).

Gampi

The bast fiber of gampi (Wikstroemia canascens) is very similar to that of mitsumata. The shrub grows wild in the mountains of Japan and is not cultivated as is mitsumata.

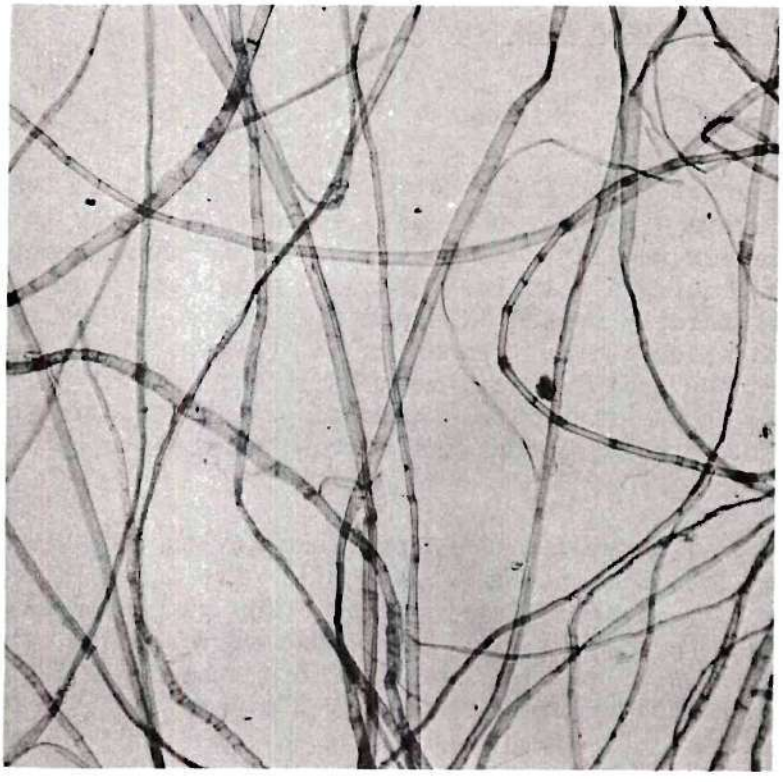


Figure 84. Kozo X100

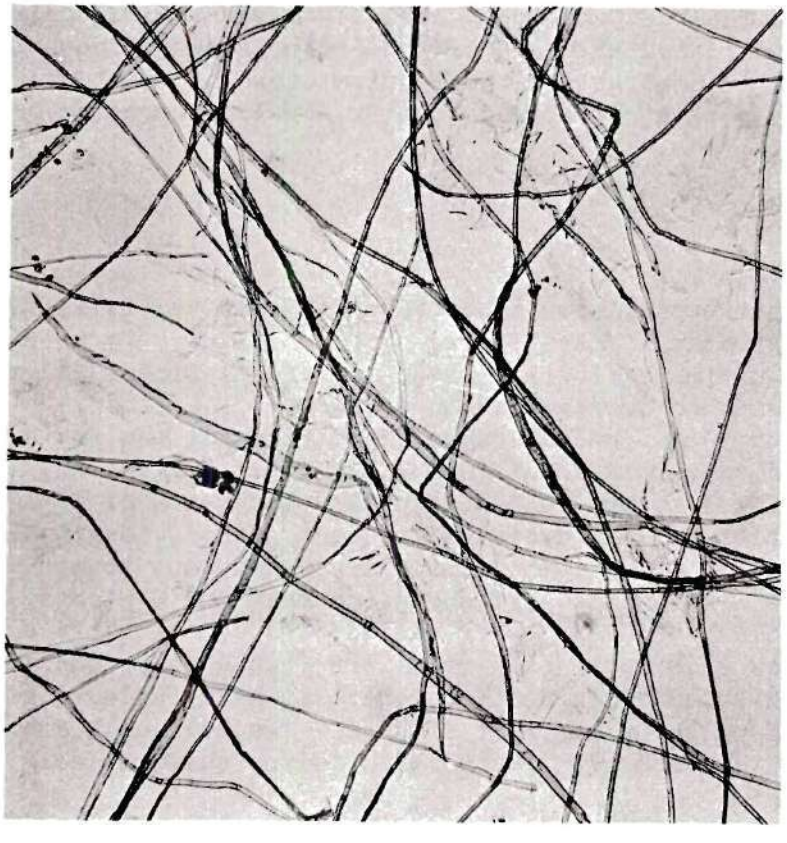


Figure 85. Mitsumata X100

Herbaceous Dicotyledon Bast Fibers

Flax

The bast fiber of flax (Linum usitatissimum) ranges in length from 6 to 60 mm., with an average length of 25 mm., and a width of 12 to 26 μm. The fibers are rather regular, being cylindrical in shape and polygonal in cross section and having a slitlike lumen. A peculiarity of the fibers is the occurrence of faintly marked "dislocations" or so-called nodes extending transversely, and often in the form of an "X" (Figure 86). These nodes are made more apparent by staining with methyl violet, or zinc chloride solution. The cross section of the flax fiber shows no yellow circumferential stain when treated with sulfuric acid, though the narrow lumen shows up as a yellow spot (3, 22, 23, 24).

The fiber from flax enters the paper industry in two forms, either as linen or directly from flax stems (Figure 87). The linen fiber as used in the textile industry is a strand of bast fibers. The strands are isolated from the other cells in the bast by a process known as retting. Textile waste, either as new cuttings or as old rags, may enter the paper industry for use in rag papers, especially currency and carbon papers.

For the past thirty years or so fibrous material for papermaking has been obtained from flax stems grown for linseed production. After running through breaker rolls, dusting, and chopping, the material (often called straw) is pulped. The resulting pulp, in addition to the bast fiber, contains other cells from the bast and woody cells from the stalk, collectively known as shives. When treated with one of the iodine stains, these short woody cells are colored blue in contrast to the reddish brown of the bast fibers. Cigaret and air-mailed papers are prepared from this pulp, where the shives do not seem to be deleterious.

Hemp

The principal use of hemp fiber (Cannabis sativa) is in the cordage industry. The strands of bast fiber, obtained chiefly by retting, are composed of the ultimate fibers. These individual fibers range from 5 to 55 mm. in length, with an average length of 20 mm. The diameter of the fiber ranges from 16 to 50 μm. and averages 22 μm. so that the ratio between the length and the diameter is about 1000. The fiber (Figure 88) is rather uneven in its diameter, and has occasional attachments of fragmentary parenchymatous tissues. It exhibits frequent joints, longitudinal fractures, and swollen fissures. The lumen is usually broad, but toward the end of the fiber it becomes like a line. It shows scarcely any contents. The ends of the fibers are blunt and very thick walled, and occasionally show lateral branches. Forked ends occur in hemp fibers, but such a condition is never observed in flax. The cross section generally has rounded edges and is not so sharp-angled and polygonal as that of jute. The cell wall exhibits a stratified appearance when the fiber is treated with iodine sulfuric acid reagent.

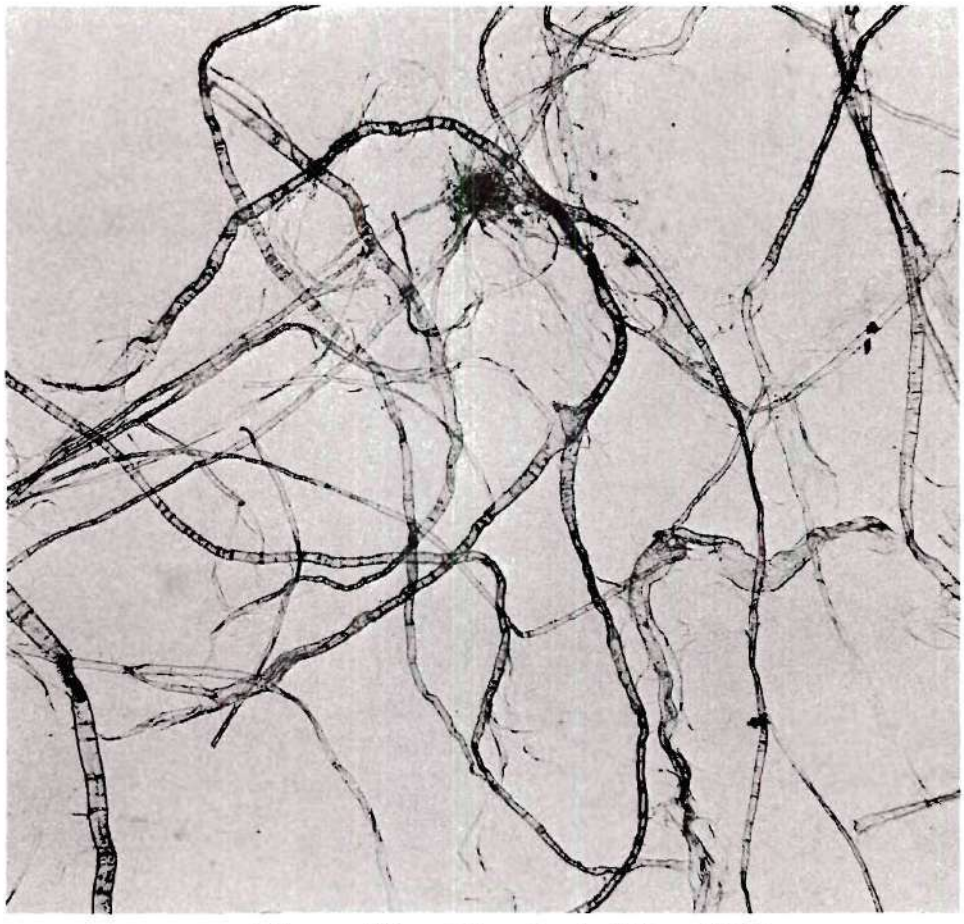


Figure 86. Flax Bast Fiber X100

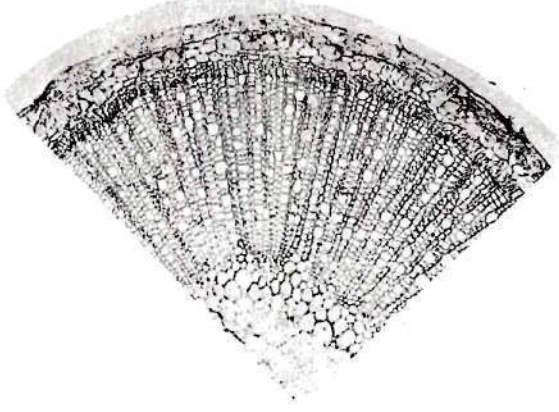


Figure 87. Cross Section of Flax Stem X50

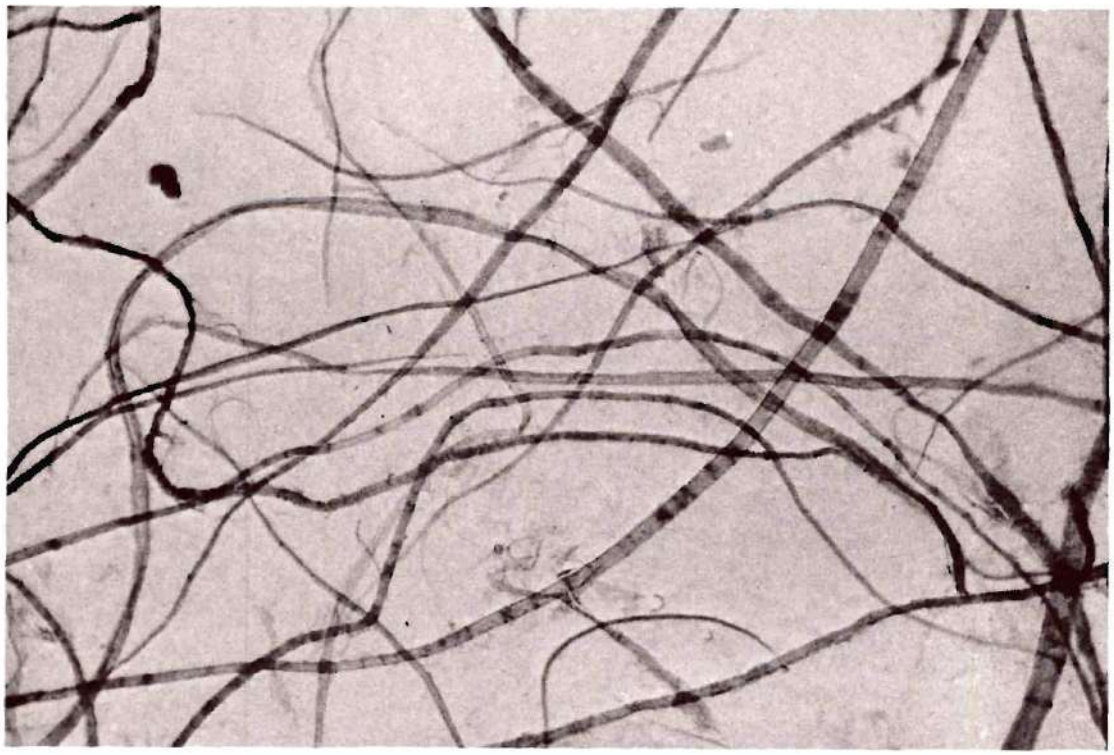


Figure 88. Hemp Bast Fiber X100

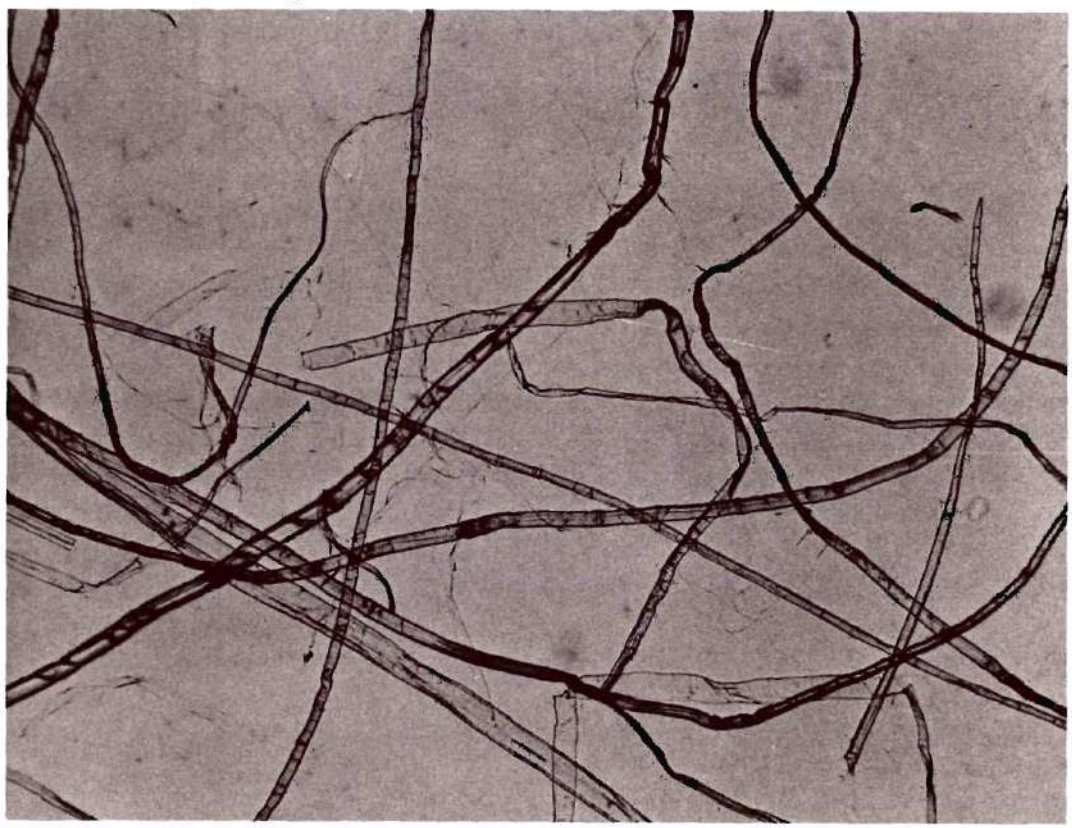


Figure 89. Sunn Fiber X100

If uncooked hemp fiber is treated with iodine sulfuric acid reagent, a green color is obtained. Hemp gives these microchemical reactions: (a) with iodine sulfuric acid reagent, bluish green coloration; (b) with zinc chloroiodide, blue or violet, with traces of yellow; (c) calcium chloroiodide, rose or red, with traces of yellow; (d) aniline sulfate, yellowish green coloration; (e) ammoniacal fuchsin solution, pale red; (f) with Schweitzer's reagent the hemp fibers swell irregularly with a characteristic appearance and dissolve almost completely, leaving only the fragments of parenchymatous tissue (3, 23, 24).

Sunn

Sunn, or Benares hemp (Crotalaria juncea), has ultimate fibers which are 2 to 8 mm. long, with an average length of 4 mm. The width is 13 to 50 μm., with an average width of 30 μm. The fibers are partly striated and also show dislocations and cross marks. The ends are thickened and either blunt or narrow with warty irregularities (Figure 89). The lumen is usually rather wide and often contains yellowish matter, differing from hemp in which the lumen is flat and narrow and usually empty. Iodine and strong sulfuric acid produce a peculiar swelling of the fibers, the outer yellow layer becoming converted into a yellow mass over which flows the blue semiliquid mass of cellulose, leaving as a residue a greenish yellow inner tube. Sunn gives a greenish blue coloration with iodine and sulfuric acid and a brownish blue coloration with zinc chloroiodide. The cross section is oval (3, 8).

Ramie

The bast fiber of ramie (Boehmeria nivea) is remarkable for its large size. The fiber length ranges from 20 to 250 mm., with an average length of 120 mm., and the fiber width is 10 to 80 μm., with an average width of 50 μm. The length:width ratio is about 2400. The diameter of the fiber is characteristically uneven, sometimes narrow, with heavy cell walls and well-defined lumen, but showing heavy striations along the fiber (Figure 90). The fiber consists of nearly pure cellulose with no indication of the presence of lignin. Iodine and sulfuric acid give a pure blue stain reaction, and aniline sulfate produces no color. In cuprammonium solution, ramie swells considerably but does not dissolve. It gives a blue coloration with zinc chloroiodide and a rose red with calcium chloroiodide. The fibers have a thick-walled, rounded point, and the lumen is reduced to a line. Joints and transverse fissures are of frequent occurrence. At places the lumen appears to be more or less filled with a granular material, evidently albuminous in nature. The cross section of the fiber is quite large, and is elliptical in shape (3, 23, 24, 25).

Jute

The individual fiber of jute (Corchorus capsularis and C. olitorius) is 1.5 to 5.0 mm. long, with an average length of 2.0 mm., and 20 to 25 μm. wide, with an average width of 22 μm. It shows no lamination either in cross section or in longitudinal view. The lumen is usually rather broad, but varies greatly in different parts of the same fiber, being in some parts

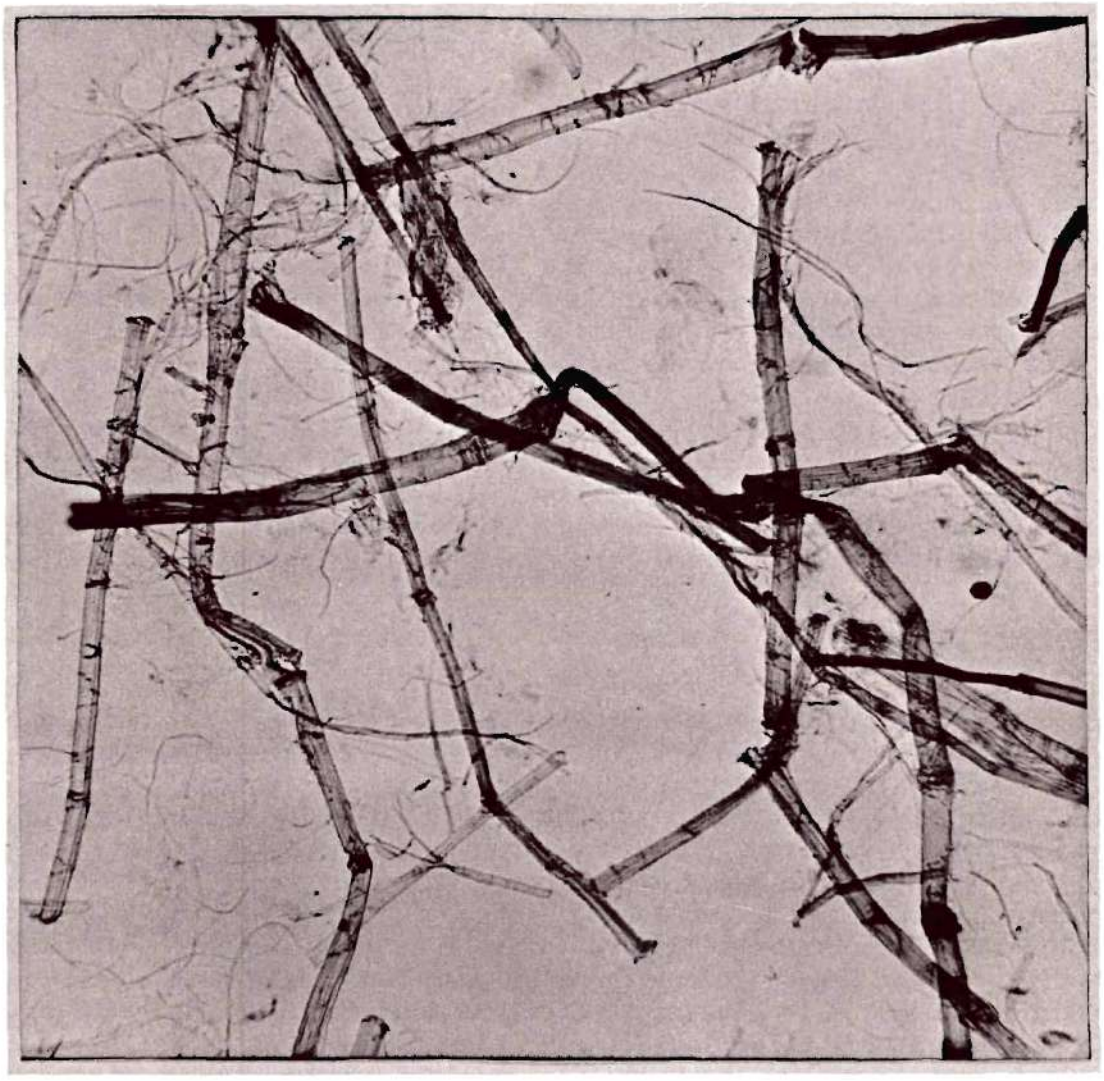


Figure 90. Ramie Fiber X100

exceedingly narrow or even closed entirely. The fiber ends are tapering to pointed. In the cross section of a fiber strand the closely united cells are polygonal, with straight sides and very sharp angles, while the lumen is either oval (where the walls are thin) or circular (where the walls are thick) (Figure 91). The cross sections are yellow throughout when treated with iodine and sulfuric acid. The exceedingly variable thickness of the fibers furnishes a valuable means for identifying the fiber; furthermore, their close grouping in bundles is of value in diagnosis. The surface of the cell is generally smooth with only occasional nodes or cross-markings. Bruises and dislocations are not present. From these remarks it is evident that jute has microscopic characteristics quite distinct from flax and hemp, but that it is similar to Bimlipatam jute (3, 23, 24, 26, 27).

Hop Vine

The bast fibers of the hop plant (Humulus lupulus) have been used in Europe to a considerable extent in the manufacture of paper. The individual fibers are 4 to 19 mm. long, with an average length of 10 mm. They are smooth, usually striated, 23 to 30 μm. wide, and for the most part have thin walls, broad lumens, and broad rounded ends, although some are narrow, very thick-walled, and pointed. Cross sections resemble those of hemp, but are mostly narrower, laminated, and after treatment with iodine and sulfuric acid display blue walls and yellow granular contents. The fibers dissolve in cuprammonium much like those of flax. Identification in pulps is exceedingly difficult as the microscopic appearance, which at best is not very characteristic, is much altered in the manufacturing process. Therefore, of considerable value are the accompanying cells, especially the so-called climbing hairs, which are unicellular, with equal or unequal hooked branches and strongly silicified walls (Figure 92) (7, 8).

Grass Fibers

Cereal Straws

There are five species of grasses which are usually thought of as the cereals, namely: rice (Oryza sativa), wheat (Triticum vulgare), oat (Avena sativa), rye (Secale cereale), and barley (Hordeum secale). Of these, wheat is used in the greatest amount in the pulp and paper industry, but all are used to some extent. Both yellow pulp, used in the manufacture of strawboard and corrugating papers, and straw cellulose, invariably marketed in the bleached state and used for making fine writing papers, are prepared from straw.

The straw pulps are composed of the sclerenchyma fibers, epidermal cells, vessel members, and parenchyma cells (Figure 93). In general, the dimensions of the various cell types of these cereal straws are similar. The typical fibers are 0.7 to 3.1 mm. long, with an average length of 1.5 mm., and 7 to 24 μm. wide, with an average width of 13 μm. The thin-walled fibers are 0.8 to 2.9 mm. long but 27 to 34 μm. wide. The slender fibers have sharp pointed ends. The other cell types are usually much shorter with a maximum length of 0.45 mm., except for the vessel members which may be as long as 1.0 mm. The cell dimensions vary somewhat with kind of straw, conditions

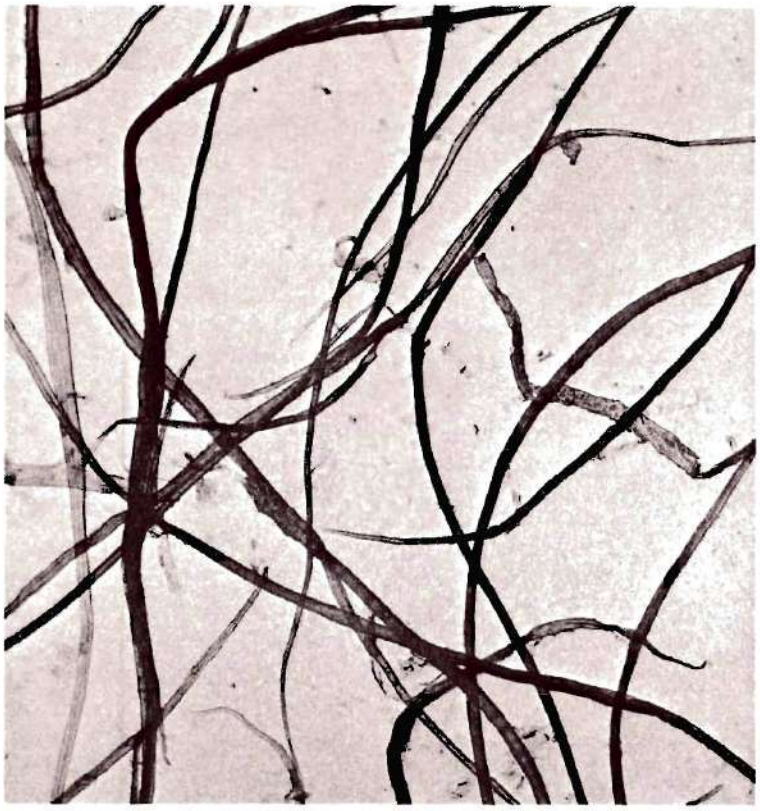


Figure 91A. Jute (X100)

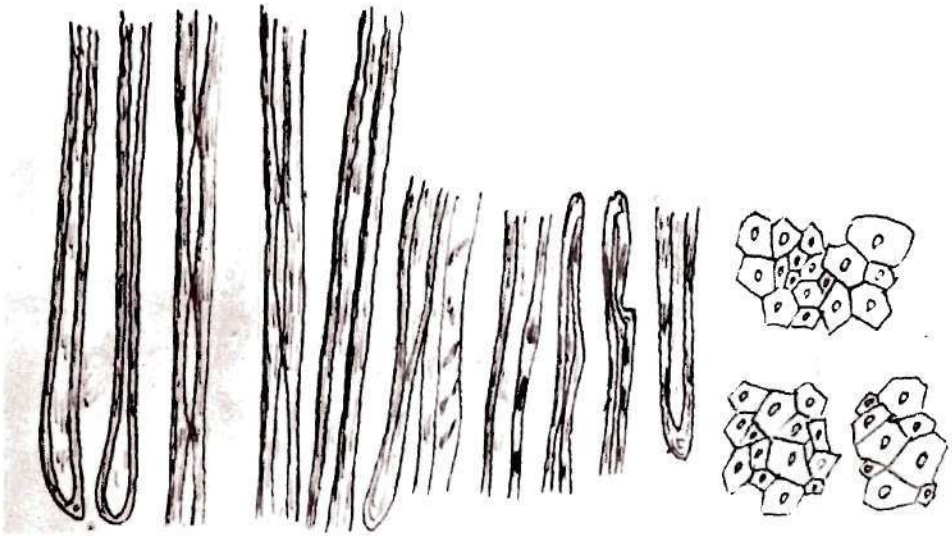


Figure 91B. Jute Fiber (Including Cross Sections of Fiber Bundles) (X290)

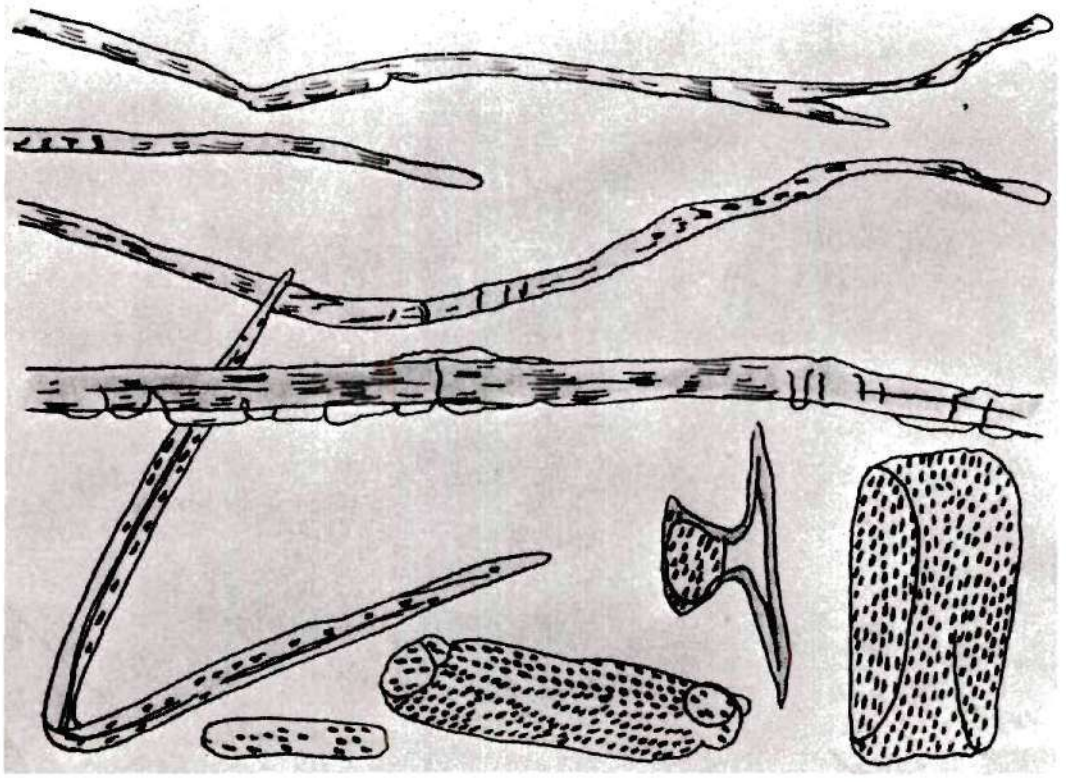


Figure 92. Hop Vine Cells X200

of growth, and nature of soil. Accompanying the fibers are numerous epidermal cells with toothed or serrated edges, and also smooth, thin-walled cells from the pithy portion of the stem (28, 29, 51).

Sugar Cane Bagasse

The fibers of sugar cane have pointed ends and are thin to thick-walled with no characteristic markings in the cell wall except for the presence of occasional small pits. The typical fiber is 0.8 to 2.8 mm. long, with an average length of 1.7 mm., and 10 to 34 μm. wide, with an average width of 20 μm. There are also comparatively short fiberlike cells with thin walls and blunt, oblique, or forked ends (Figure 94).

The separated thick rings of the primary vessels, which occur only rarely in bamboo pulp, are frequently found. The pitted vessels have two forms: very broad ones having narrow, parallel rows of pits which are quite similar to those of bamboo; also, much narrower vessels having larger holelike pits which are lacking in bamboo. The vessel segments have a maximum length of 1.35 mm. and a maximum width of 150 μm. Large, thin-walled, finely pitted parenchyma cells also occur fairly abundantly. Their maximum length is 850 μm. and their maximum width is 140 μm. Rectangular epidermal cells with serrated margins are also present (28, 30, 31).

Figure 93. Rice Straw Pulp X100

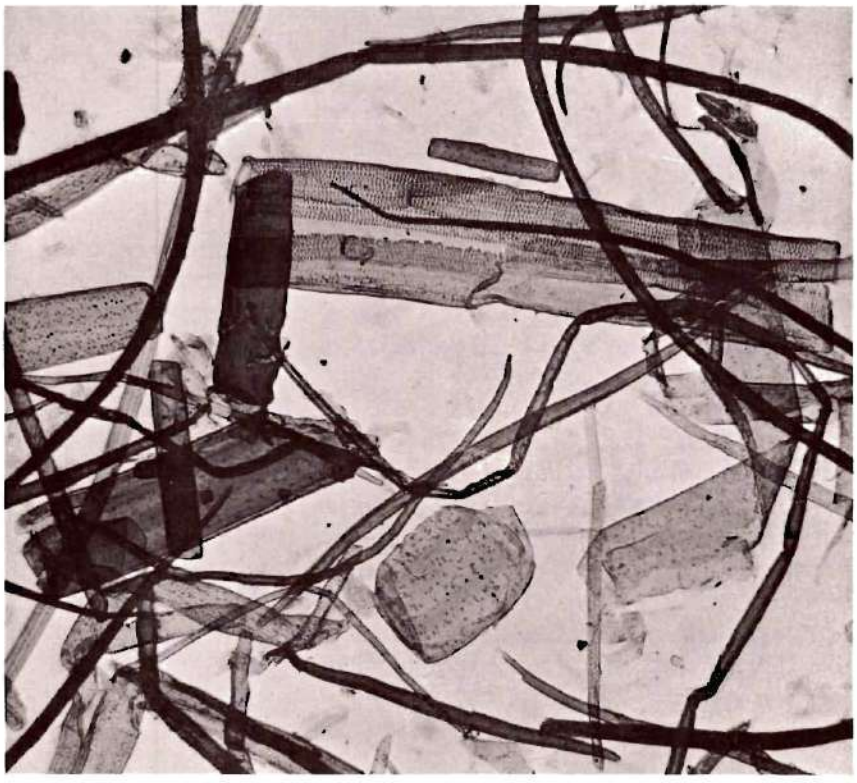


Figure 94. Bagasse Pulp X100

Cornstalks

The various cells of cornstalks (Zea mays) are not readily distinguishable from bagasse or the cereal straws. The typical fibers are 0.5 to 2.9 mm. long, with an average length of 1.5 mm., and 14 to 24 μm. wide, with an average width of 18 μm. The shorter, thin-walled, pitted fibers average 0.44 mm. in length and 16 μm. in width. The pitted vessel segments have a maximum length of 1.35 mm. and a maximum width of 150 μm. The saclike, thin-walled parenchyma cells have a maximum length of 325 μm. and a maximum width of 150 μm. Rectangular epidermal cells with serrated margins occur in the pulp (5, 7).

Bamboo

There are more than 200 species of bamboo in several genera. The stems of some species are only a few inches tall but others are 120 feet in height. The enormous quantity of bamboo in the world, and its very rapid growth, makes this peculiar grass a promising source of papermaking material. Considerable amounts of bamboo are now used for papermaking in India and other countries of southeast Asia. Among the species used in the manufacture of pulp and paper are: Bambusa arundinacea, B. tulda, B. balcooa, Dendrocalamus strictus, Melocanna bambusoides, Pseudostachyum polymorphum, Teinostachyum dulloa, and Thyrostachys siamensis. The typical fibers found in these species are 1.5 to 4.4 mm., mostly 2.2 to 2.6 mm., in length, and 7 to 27 μm. in width, with an average width of 14 μm. The wide, thin-walled, pitted fibers are 2.8 to 3.2 mm. long and 20 to 40 μm. wide. The pitted vessel segments have a maximum width of 100 μm. Numerous serrated epidermal cells and pith parenchyma cells are also present. The pith cells have a maximum length of 250 μm. and a maximum width of 65 μm. (Figure 95) (28, 32, 32a).

Esparto

Two species (Stipa tenacissima and Lygeum spartum) of closely related grasses are the source of the esparto (alfa, halfa, sparto) fiber of the paper industry. The grasses gow in Algeria, Morocco, Tunisia, Tripolitania, and Spain. In Algeria, Stipa grows best in sandy, ferruginous soils in dry, sunny situations on the sea coast while Lygeum is found in rocky soils on the high plains of the interior.

Esparto fibers are among the slenderest encountered in the paper industry with an average fiber width of 7 μm. and a range from 4 to 11 μm.; the lumen is narrow. In length the fiber ranges from 0.5 to 2.5 mm., with an average length of 1.5 mm. Serrated epidermis cells are numerous but are considerably smaller than those from straw, while the smooth, thin-walled cells are absent. The chief characteristic which distinguishes esparto from straw and other fibers is the presence of small tear-shaped cells derived from the stiff hooked hairs on the surface of the leaf (Figure 96). Some authors claim there is a difference between both the fiber diameter and the shape of these hair cells in Lygeum as compared with Stipa. Such a difference, if it exists, does not seem readily detectable. The fiber of Lygeum is supposed to have a distinct lumen and be correspondingly broader. The hairs in Lygeum are supposed to be provided with a wide lumen, are generally slightly curved but never hooked (33).

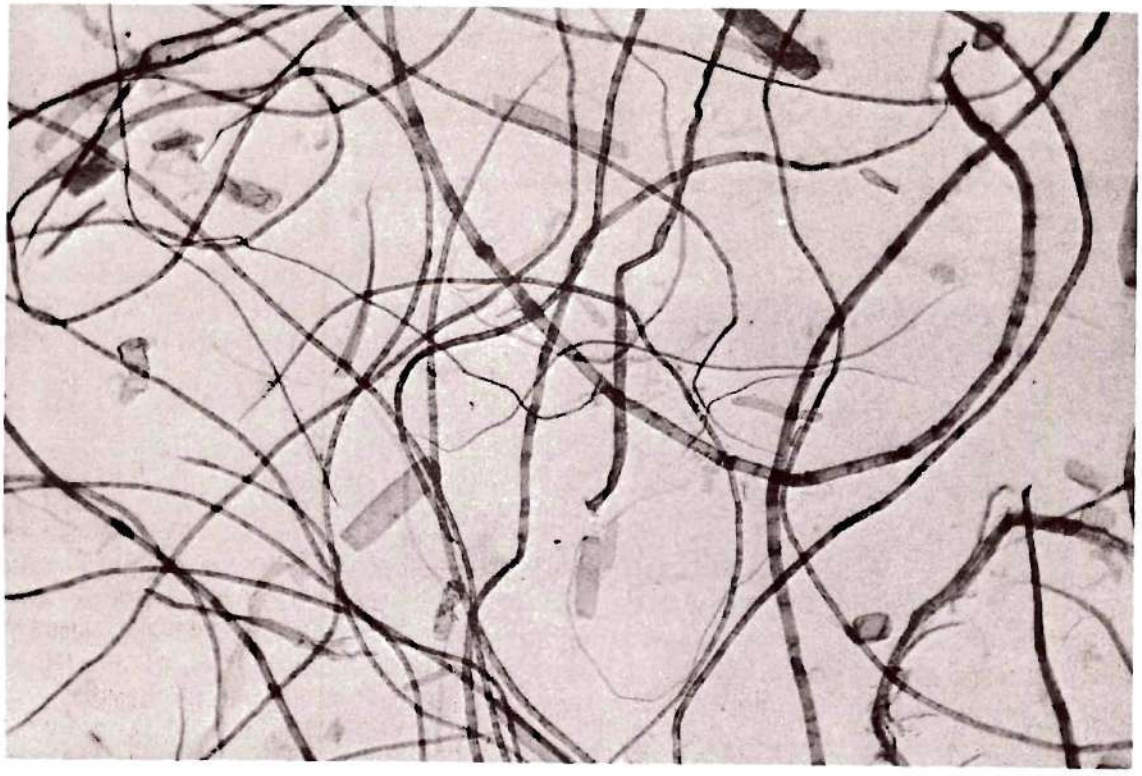


Figure 95. Bamboo Pulp X100

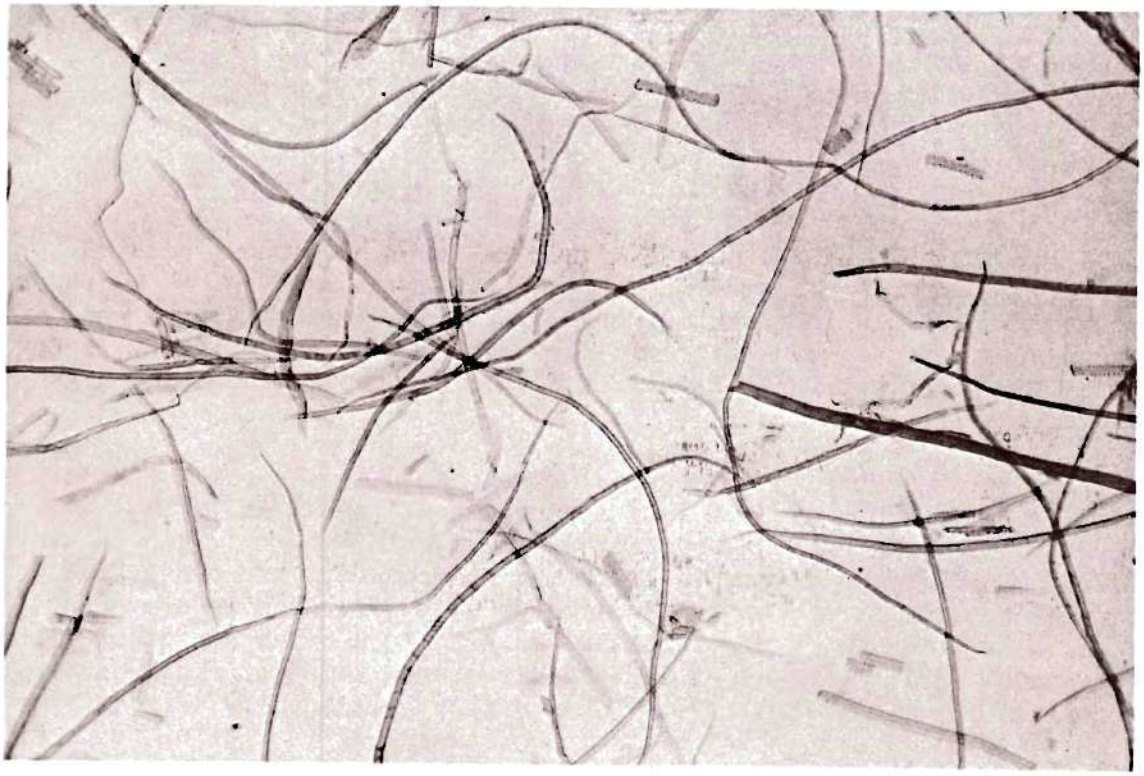


Figure 96. Esparto Pulp X100

Sabai

The pulp of sabai grass (Eulaliopsis binata) resembles that of rice straw and millet (Figure 97). The fibers appear to be grouped according to length with short ones up to 1 mm., and long ones generally 2.0 to 2.5 mm., with a maximum length of 4 mm.; thus, they are much longer than the fibers of rice straw. The fiber width is 9 to 16 μm. The fibers are very uniform, have very thick walls, and a very narrow lumen. The ends are generally pointed and never forked. The epidermal cells differ from those of rice straw owing to the lack of bosslike protuberances (34).

Leaf Fibers

Abaca, or Manila Fiber

Individual fibers of abaca (Musa textilis) range from 2 to 8 mm. long, with an average length of 4 mm., and 16 to 32 μm. in width, with an average width of 24 μm. The fibers taper very gradually toward the ends. The fiber is rather thin-walled and the lumen is large and rather distinct. The cross sections are irregularly round or oval in shape. Numerous fine cross markings are evident on many of the fibers (Figure 98).

Manila fiber is one of the most important fibers used in cordage. Much of the material which enters the pulp and paper industry is in the form of old rope. Virgin fiber obtained directly from the source is also used in the paper industry. The strands of fiber as isolated from the stalks are very large, but by treatment with alkali they are easily separated into smooth, even fibers. The fiber bundles frequently show a series of peculiar, thick, strongly silicified plates, known as stegmata. Lengthwise these appear quadrilateral and solid; they have serrated edges and a round, bright spot in the center. The stegmata may be observed best after the fiber bundle has been macerated; they are about 30 μm. long. Extraction of the fiber with nitric acid, ignition, and addition of diluted acid to the ash causes the stegmata to appear like a string of beads, a very peculiar and characteristic appearance (3, 35, 36).

Sisal

The fibers of sisal (Agave sisalina) are 1.5 to 4.0 mm. long and 20 to 32 μm. wide, with an average width of 24 μm. The fiber walls are thick, characterized by many fine cross lines, and the lumen is not prominent. The fiber ends are broad, blunt, and thick, and at times forked (Figure 99).

Filaments of fibers are light-colored, lustrous, and comparatively stiff. The ash obtained from the ignition of the fiber shows the presence of glistening crystals of calcium oxalate to be found clinging to the fiber bundles. The occurrence of these crystals is very characteristic of these fibers. Iodine and sulfuric acid treatment colors the fiber yellow (3, 37).

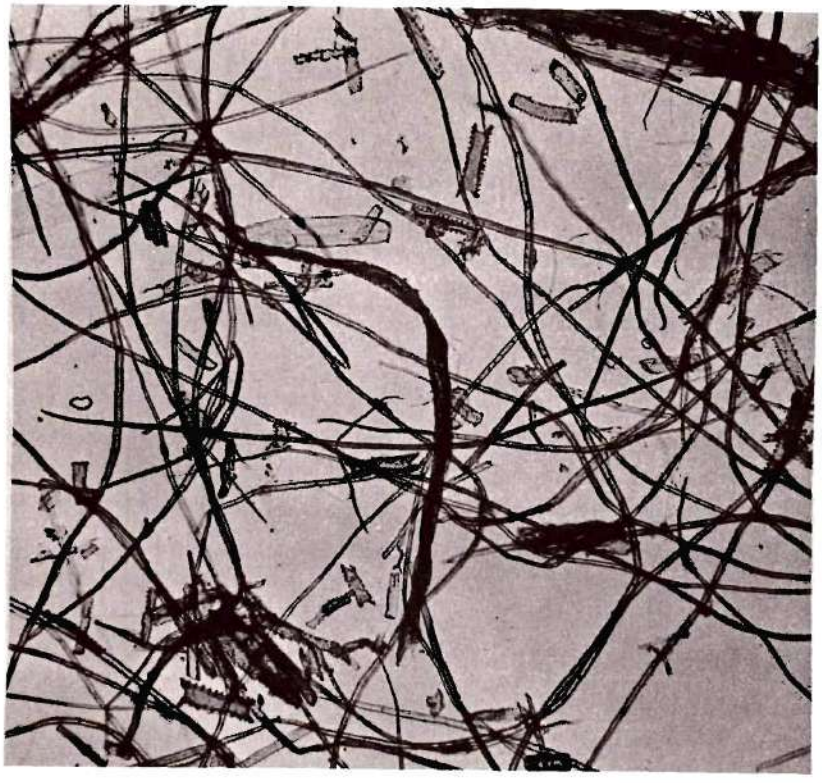


Figure 97. Sabai Grass Pulp X100

Other Agave fibers are used for purposes similar to sisal and possibly enter the paper industry as waste material. The principal species is henequen, Agave fourcroydes, produced chiefly in Yucatan, Cuba, and El Salvador.

Phormium, or New Zealand Flax

The fibers of phormium (Phormium tenax) are 5 to 15 mm. long, with an average length of 8 mm., and 10 to 20 μm. wide, with an average width of 16 μm. They are very regular and uniformly thickened, and the surface is smooth, though occasionally exhibiting wavelike irregularities in the cell wall. The lumen is very apparent, but is generally narrower than the cell wall and is very uniform in width. The ends taper to a moderately narrow point (Figure 100A). In cross section the fibers show rather loosely adhering elements and are very round in contour (Figure 100B). The lumen is either round or oval and is empty. Fragments of parenchyma and epidermis are frequently noticed on the fibers. No median layer of lignin is apparent between the elements, though the fibers are lignified (38, 39).

With iodine and sulfuric acid, the fibers exhibit an intense yellow color, with aniline sulfate a pale yellow, with zinc chloroiodide a yellowish brown, with ammoniacal solution of fuchsin, a red; with Schweitzer's reagent the fibers are rapidly separated into their elements, but they do not dissolve. The purified phormium fiber is rather difficult to distinguish from sansevieria fiber, except by the rounded and separate cross sections.

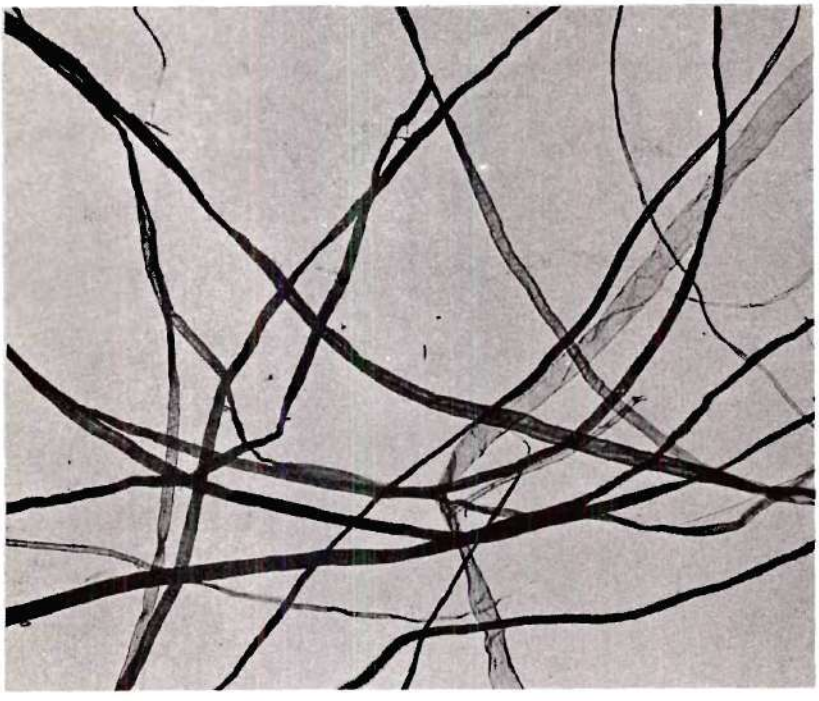


Figure 98A. Abacá Pulp X100

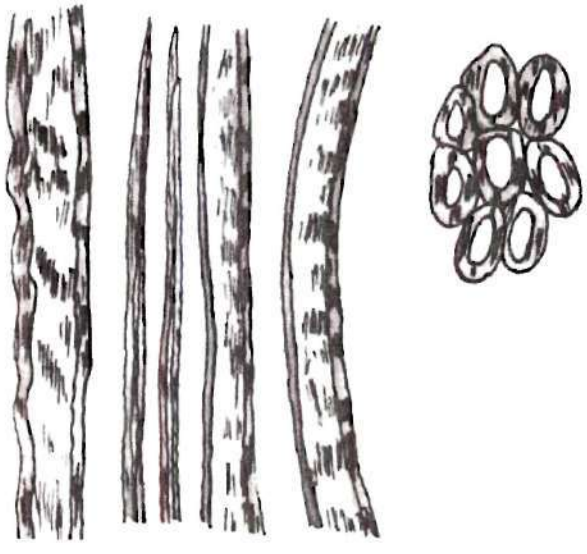


Figure 98B. Abacá Fiber (Including Cross Section) X400

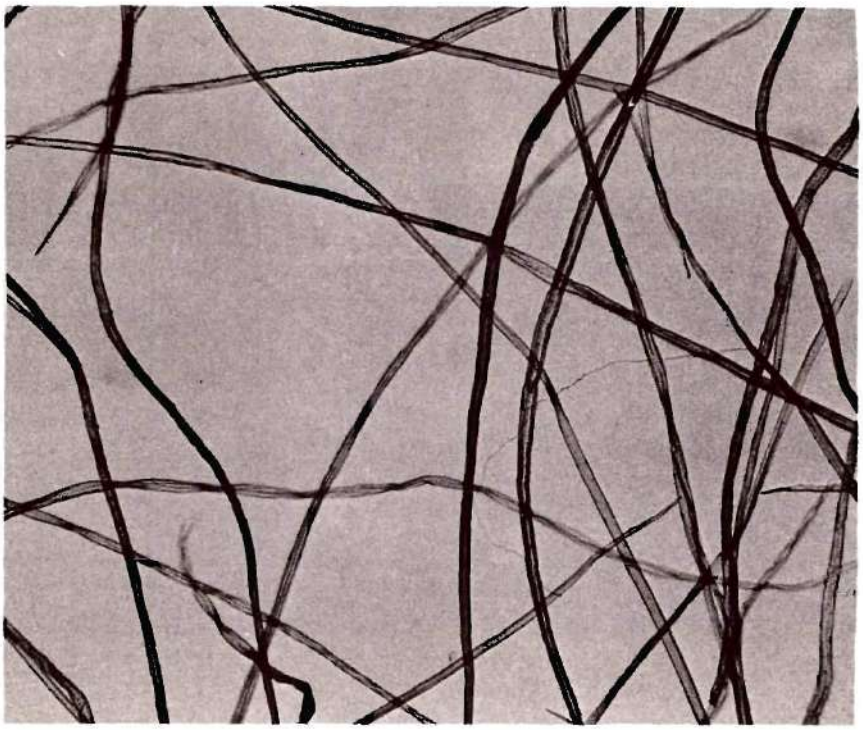


Figure 99A. Sisal Pulp (X100)

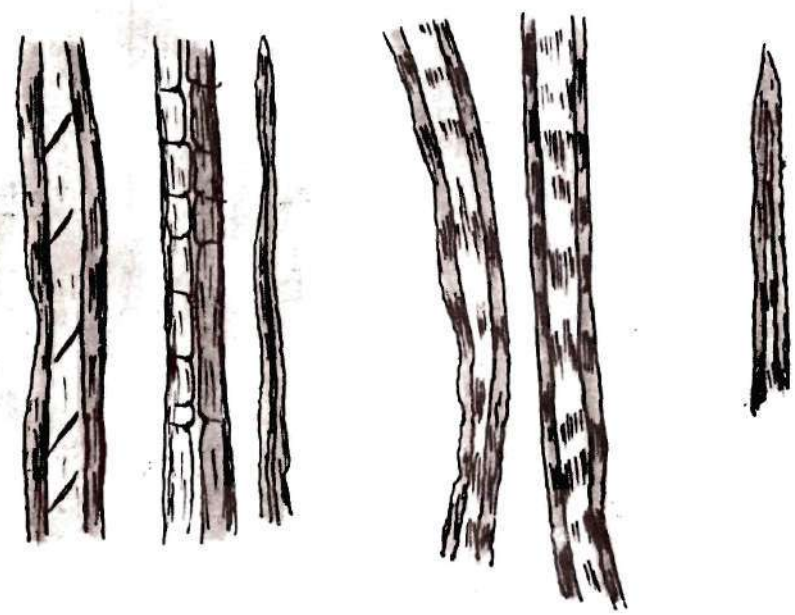


Figure 99B. Sisal Fiber (X300)

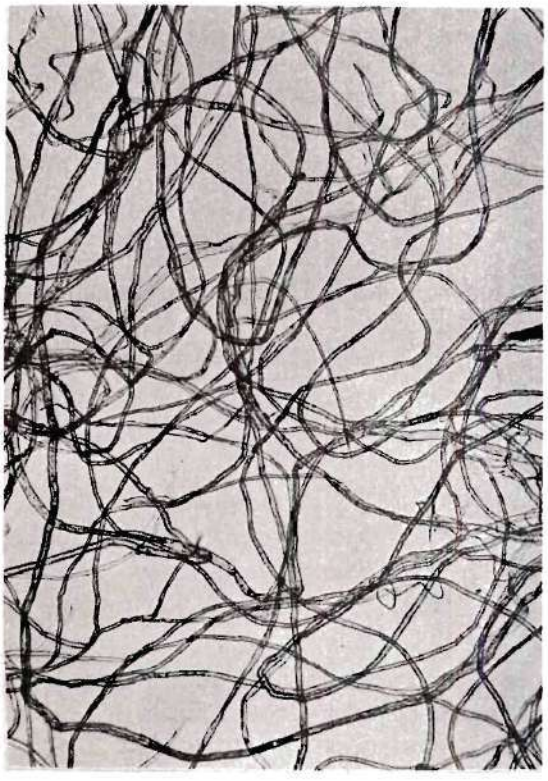


Figure 100A. Phormium Pulp X100

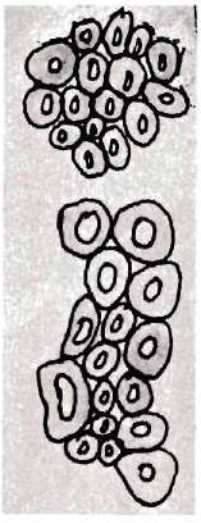


Figure 100B. Cross Section of Phormium Fiber Bundles X300

Pineapple

The pineapple fiber (Ananas comosus) is distinguished from other leaf fibers by the extreme fineness of its fiber elements. The fibers are 2.8 to 8.9 mm. long and 4 to 8 μm. wide. The lumen is very narrow and appears like a line. The cross sections are polygonal in outline and frequently flattened. The sections form compact groups, which are often crescent shaped, and are enclosed in a thick median layer of lignified tissue. The fibers are accompanied by vascular bundles in which there frequently occur several rows of thick and strongly lignified fibers (3, 40).

Pita Floja

The pita floja fiber (Aechme magdalenae) closely resembles the pineapple fiber in appearance. The fibers measure 1.5 to 6.1 mm. in length and 8 to 16 μm. in width which is nearly twice that of the pineapple fiber and which serves to distinguish it from pineapple fiber (3, 40).

Caroa

The caroa fiber (Neoglaziovia variegata) resembles the other members of this group, pineapple and pita floja. The fibers are 2 to 6 mm. long, with an average length of 4.1 mm., and 8 to 12 μm. wide. This species grows in Brazil and is used for fiber purposes and in the paper industry (3, 40).

Mauritius Fiber

The fiber of this species (Furcraea gigantea) is 1.3 to 3.7 mm. long and 15 to 24 μm. wide (3).

ANIMAL FIBERS

Silk Fibers

Cultivated Silk

When raw silk, spun by the silkworm (Bombyx mori), is examined under the microscope it exhibits features which readily distinguishes it from other textile fibers (Figure 101A). The longitudinal view shows a very irregular surface structure, mostly in the sericin layer, which consists of traverse figures, creases, folds, and uneven lumps. These markings are largely due to the reeling operation when the soft gum is slipped or broken in the crossing or croissures. Frequently, the two fibers of a cocoon filament are distinctly separated from one another for considerable distances, the intervening space being filled in with sericin. All of these markings are in no ways structural, and only occur in the sericin layer.

The cross section of a cocoon filament is roughly elliptical, showing the two triangular brins completely surrounded by sericin normally facing each other with the flat side of the triangle. The ellipticity of the cross section varies from the outside which is nearly circular to the inside of the cocoon which is usually very much flattened.

The longitudinal view of degummed fiber shows a smooth, structureless, translucent filament with occasional constrictions as well as swellings or lumps (Figure 101B). The fiber is rarely striated longitudinally but when such striations do appear they always run parallel to the fiber axis; and when such fibers are treated with dilute chromic acid, very fine striations appear (3).

Tussah, or Wild Silk

The cross-sectional contour of tussah silk, from the wild silkworm (Antheraea pernyi), is definitely wedge-shaped (Figure 102). In the raw silk, the two small sides of the wedge face each other and are surrounded by the silk glue. The filament structure can be recognized easily in the cross section by the grainy inner structure of the fiber and the sawtoothlike contour

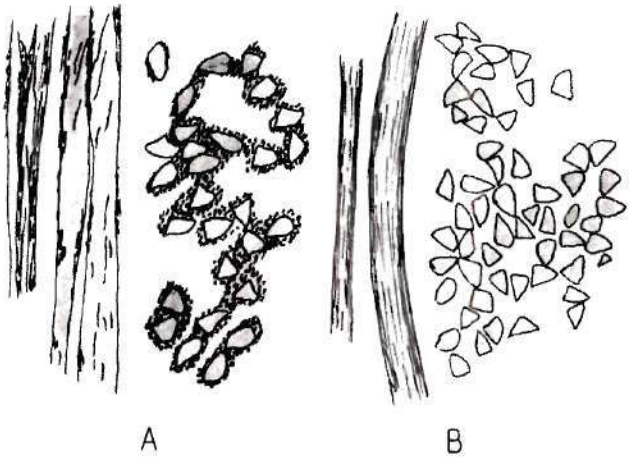


Figure 101A. Raw Silk, B, Degummed Silk
X200

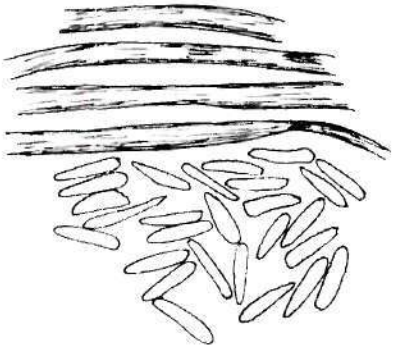


Figure 102. Tussah Silk
X200

of some of the fibers. Taken collectively, wild silks are similar in their microscopic structure and it is difficult to differentiate among the various species. They are distinguished from cultivated silk in that they are darker in color, have a ribbonlike form, and are strongly fibrillous, with a wedge-shaped cross section (3).

Wool

Wool is an animal fiber forming the protective covering of sheep. As a product of the skin or cuticle of the vertebrate animals, it is similar in origin and general composition to the various other skin tissues found in animals, such as horn, nails, and hoofs. Wool is an organized structure, growing from the root situated in the middle layer of the skin (3, 41).

Increase in fiber length is brought about by the proliferation of new cells in the root and the subsequent emergence of these cells into the shaft. The dead cellular units composing the shaft form three distinct regions: a thin outer covering, the epidermis or cuticle; a middle region, the cortex; and an inner central core, the medulla (Figure 103A).

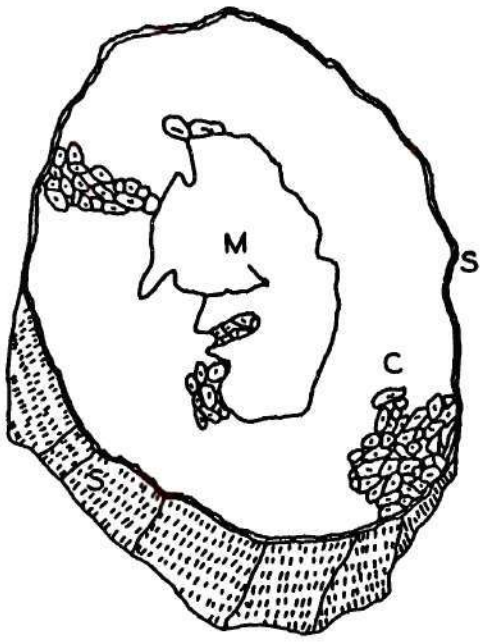


Figure 103A. Wool Fiber Cross Section
M, Medulla; C, Cortical Cells; S, Scales or Epidermis (After McMurtrie)

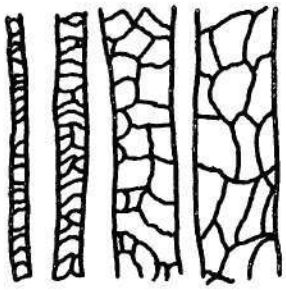


Figure 103B. Various Types of Wool Fiber Scale Structure

The outside or surface of the fiber is made up of flat irregular horny cells or scales. They overlap like the shingles of a roof with the free end projecting outward and pointing toward the tip of the hair, causing the surface of the fiber to present a serrated appearance (Figure 103B). The average height of the scales is about 28 μm. and the average width is about 36 μm. The number of scales visible on the edge of the fiber ranges from 4 to 12 per 100 μm. for fine and medium wools. The thickness varies between 0.5 and 1 μm. The scales form a pattern on the surface of the fiber the complexity of which varies not only from fiber to fiber but also upon one fiber.

The cortex constitutes the principal body of the wool fiber and is made up of long, slightly flattened and more or less twisted, spindle-shaped cells. The average cells are 80 to 110 μm. in length, 2 to 5 μm. in width, and 1.2 to 2.6 μm. in thickness. These cells are composed of many fibrils and show longitudinal striations when mounted in water.

In medium and coarse wools, a third layer is found within the cortical layer, a cellular marrow or medulla. The medulla is built up of many superimposed cells, of various shapes, often polygonal, forming a honeycomblike structure. The presence of medullated fibers in any wool is detrimental to quality from the standpoint of the manufacturer.

The diameter of wool fibers varies greatly, even in the same fleece; it may range from 10 to 70 μm. The shape of the cross section varies greatly also, from nearly circular to a varying degree of ovality or ellipticity. Wool fibers grow in a more or less wavy form and with a certain amount of twist. The length of the fiber varies in large limits.

Chemically, the wool fiber is classed as a protein called keratin. Wool when burned gives off a characteristic odor, similar to that of burning hair or feathers, due chiefly to the presence of nitrogen in the fiber. One of the most characteristic chemical properties of wool is the ease with which it is degraded in alkaline solutions. A five percent solution of caustic soda at boiling temperature completely dissolves wool in a few minutes.

Specialty Hair Fibers

In addition to the fiber obtained from the various types of sheep, large quantities of animal fibers not strictly classified as wool are used in the manufacture of clothing. These fibers are obtained from goats, camels, cows, horses, and fur-bearing animals. Should the analyst encounter fibers which he suspects belongs to this category he is referred to detailed microscopic information as such details are beyond the scope of this chapter (3, 45).

Leather Fibers

Originating from the hides of animals, leather consists of fibrous tissue, which, broadly speaking, can be said to exist in the form of bundles intimately connected and interlaced with one another.

If a small portion of board containing leather is boiled in a 0.5% solution of potassium hydroxide, the solution not only gives off a distinguishing odor similar to that of stewed meat, but gradually becomes dark greenish brown to red in color, because of the reaction between the reagent and the tanning materials still present in the leather.

After treatment in the caustic potash the board is easily disintegrated, and if a portion is transferred to a microscope slide and covered for observation, the animal tissues will be seen readily through the microscope as swollen, golden red to deep brown masses or irregularly shaped objects, interspersed between the typical vegetable fibers (if any of these are present).

Because of the preliminary treatment given to the scrap leather in the preparation of leather boards many pieces of animal tissue almost completely lose their characteristic bundle formation during the caustic treatment, the individual fibers coalescing. It is therefore advisable to thoroughly compress the cover slip over the specimen, giving it a slightly sliding motion so that the individual specimens of animal tissue are squashed and somewhat disintegrated.

The typical striations of muscular tissue can usually be seen in isolated places after this dissecting by compression (42).

MINERAL FIBERS

Asbestos Fibers

Single fibers of asbestos as seen under the microscope are very straight, resembling a "finely polished metal rod" without any rough surfaces as is the case with organic and vegetable fibers (Figure 104). Therefore, when spun they have a tendency to slip past each other with little friction, making the spinning of asbestos a difficult matter if no vegetable fiber is blended with them.

Photomicrographs of chrysotile and crocidolite (blue) asbestos fibers show a decided difference between these two types, and easily explain why chrysotile asbestos is somewhat easier to spin than crocidolite. Chrysotile fibers are "curly" and have a greater tendency to cling together in yarn formation than the straight blue fibers.

All asbestos fibers, when examined under a lens, show the same characteristics of crowding or grouping together of numerous fine threads within what appears to be a single fiber. The actual size of the fibers, that is, the diameter, has never been definitely determined, because even the finest filament ever measured is itself composed of fine threads.

The chrysotile asbestos fiber contains magnesium silicate and comes from Canada or Russia, and the crocidolite fiber contains iron silicate and comes from South Africa or Australia (3, 52).

MAN-MADE OR ARTIFICIAL FIBERS

Glass Fibers

Glass fibers are readily distinguished from all other textile fibers on close examination, but not on casual examination of glass textiles. Careful examination and simple flame or stretch tests readily reveal essential differences.

Microscopic examination of glass fibers (washed clear of any lubricant or binder) reveals that they are perfectly smooth, with no visible structure on the surface (Figure 105). The edges of the fiber are always parallel and without any irregularities whatever. Even study under the electron microscope fails to reveal any roughness or irregularity.

In cross section the fibers are perfectly circular. Fiber diameters may vary considerably from the average dimension, but these variations are not significant until "tramp" fibers considerably coarser than the average appear. The finer fibers merely tend to increase breaking strength and flexibility; hence, quality control standards merely seek to maintain an average fiber diameter within 1.23 μm. of the specific normal fiber size and to reject only when the largest fiber present exceeds the average by 2.50 to 3.80 μm.

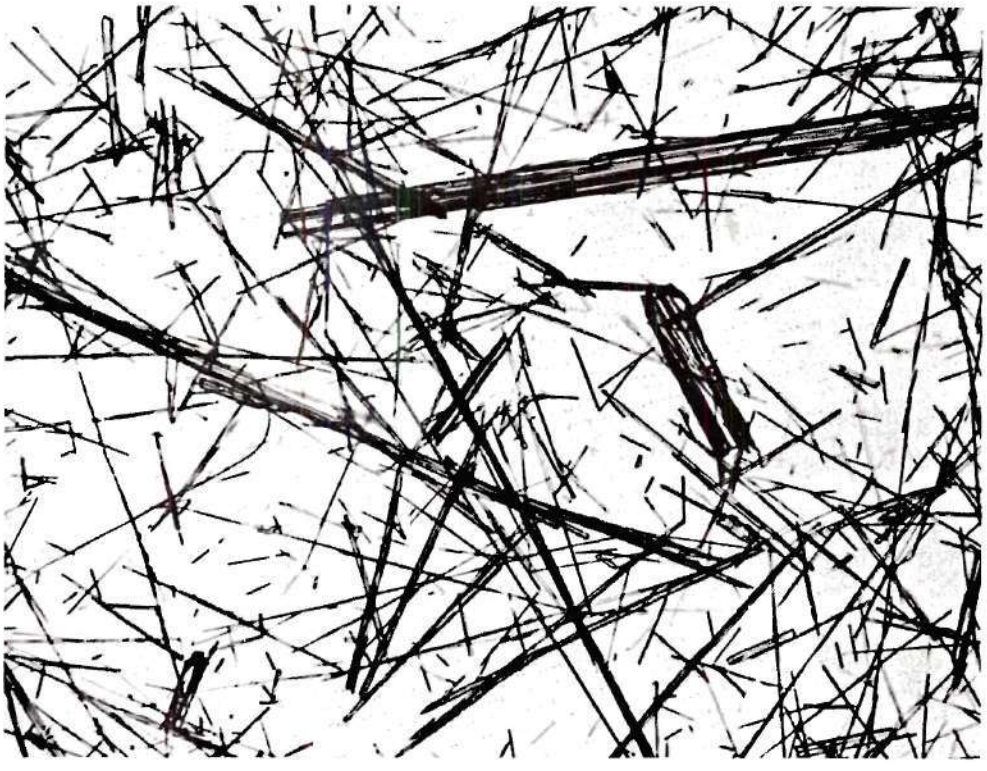


Figure 104. Asbestos Fibers X100

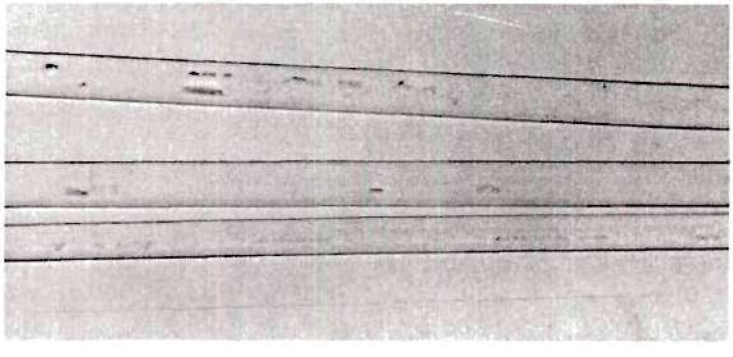


Figure 105. Glass Fibers X300

The characteristic fracture of a glass fiber is a clean transverse or slightly diagonal break. The fibers never, or rarely, split or break longitudinally. Breaks are caused in fibers by the same stresses that break sheet glass or larger rods; if the surface is nicked by abrasion of glass against glass, or by any hard instrument, the fibers are weakened at that point just as a glazier's cutting tool causes sheet glass to break cleanly under the scratch on the surface. Similarly, a fiber is broken as any larger rod or sheet of glass is broken, simply by bending it sharply (3, 43, 52).

Regenerated Cellulose Fibers

Viscose Rayon

Viscose rayon filaments exhibit special microscopic characteristics. The medium for mounting regenerated cellulose rayons, that is, viscose and cuprammonium, is glycerin (n = 1.46) or monobromonaphthalene (n = 1.66). Microscopic examination is valuable and indispensable for identification of such fibers in blends, for width measurements and denier determination, process of manufacture, and degree of dullness (3, 13, 44).

The longitudinal examination is best made at magnifications of 250 to 500 diameters and with monobromonaphthalene as a mounting medium. This brings out a number of channels or striations parallel to the fiber axis, produced by the shrinking of the filament after it leaves the spinneret. These are more visible in bright than in dull filaments (Figure 106A). They are particularly clear and sharp at 500 magnification and may vary in a random manner. Therefore, they cannot be used for identification purposes or for determining the origin of any viscose rayon filament. Dullness is readily ascertained by scattered black specks both in longitudinal and cross-sectional view (Figure 106C).

The cross section of viscose rayon filaments and staple fibers is particularly characteristic and valuable for identification. In fact it is the only positive means. The cross section may vary in size, according to the denier, and may vary greatly in outline and shape. The major factors responsible for its shape are the nature and strength of the coagulating bath and the composition and age of the viscose solution. The size of the spinneret hole and the amount of stretching that is given during and after coagulation have only a minor effect. The chief distinguishing characteristic is the strongly serrated contour or outline.

The section may vary in outline from almost round or circular, irregular, or oval to flat or ribbonlike. The indentations in the viscose rayon cross sections are irregular, sharp, numerous, and not very deep. To get a sharp outline it may be necessary to dye the filaments lightly, because the dye will penetrate completely to the inside. Dyeing is particularly advantageous to show the difference between the setting of the outside and the inner part of some types of viscose (Figure 106B).

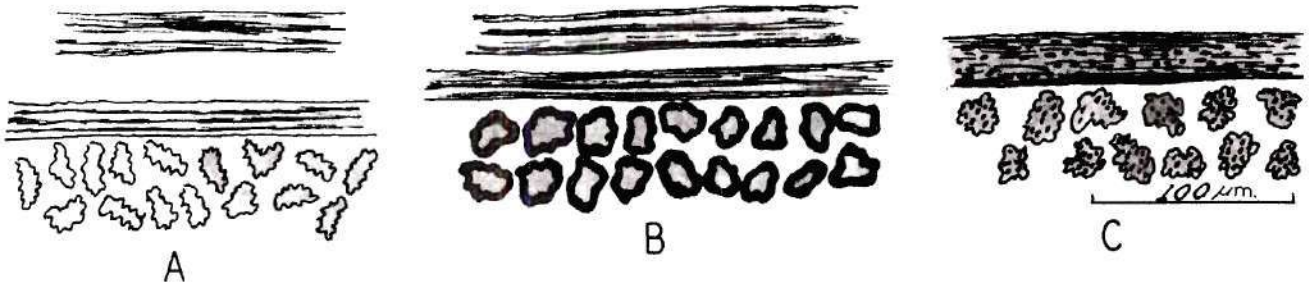


Figure 106. A, B, C. Viscose Rayon Filaments X200

Cuprammonium Rayon

In longitudinal view the filaments of cuprammonium rayon appear fine and structureless, without striations or markings of any kind (Figure 107). In cross section the fibers are circular or sometimes oval, with a smooth contour, which is very characteristic of this rayon (3, 13, 44).

Cellulose Ester Fibers

Acetate Rayon

Under the microscope, cross sections of regular commercial cellulose acetate rayon filaments or fibers are revealed as having two, three, four or occasionally more, smoothly rounded lobes (Figure 108) which are readily distinguishable from the serrated edges of cross sections of the viscose rayons (Figure 106). In lateral view, the fiber surface is smooth and the lobes are visible as gently rounded longitudinal ridges and valleys. Bright luster acetate rayon is clear and transparent. In dull luster fibers the pigment particles can be seen as tiny specks, distributed throughout the fiber substance. The dimension of the pigment particles is so small in comparison to the width of the fibers that even those particles that lie in or near the fiber surface do not appreciably affect the smooth contours of the surface. The general shape of the acetate rayon cross section is much the same for fine or coarse fibers.

When a filament of acetate rayon is examined by polarized light, a longitudinal view shows only dark, first order gray polarization colors, which may be almost invisible. However, when a "first order red" plate is inserted between the crossed nicols, the fibers appear purple and orange against a red field.

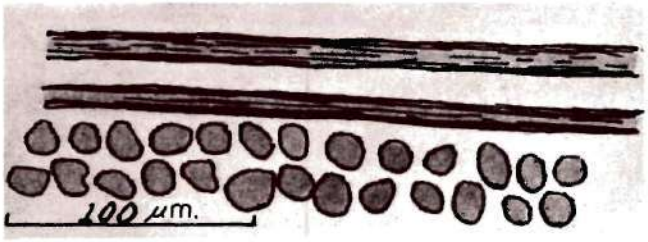


Figure 107. Cuprammonium Rayon X200

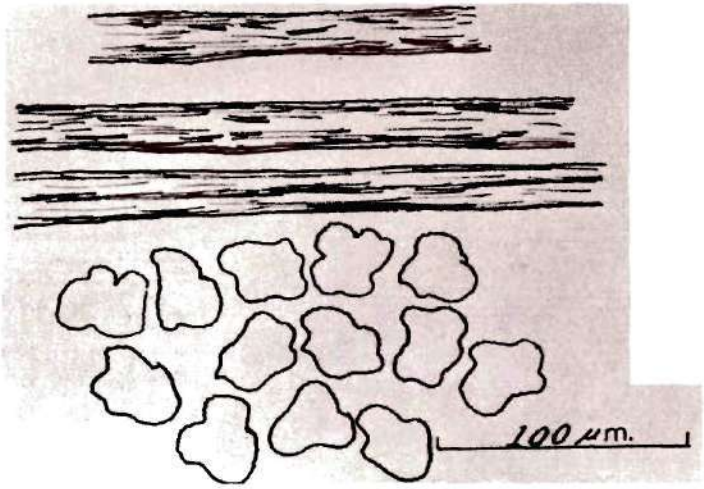


Figure 108. Acetate Rayon X200

A cross section illuminated with plane polarized light will appear light in some places and dark in others. When the plane of polarization is rotated 90°, the dark places become light and the light places become dark. This phenomenon is due to the fact that the micelles in the fiber appear dark when they are at right angles to the axis of the fiber and lie in the plane of vibration of the light.

Acetate rayon of the usual type can usually be identified readily by microscopic examination because of the characteristic shape of the cross sections (3, 13, 44).

Protein Base Fibers

Casein

According to Moncrieff (47) the casein fibers being produced commercially in 1963 were Fibrolane in England, Lanital in Belgium, Merinova in Italy, and Caslen in the U. S.; Aralac is no longer produced.

Microscopically, the domestic fiber differs little from Lanital. In longitudinal view, the fiber shows characteristic faint striations and a grainy surface, particularly the bright fibers. The difference between the bright or natural and the pigmented or dull fiber is very marked. The cross section is highly circular, but the contour is perfectly smooth, with no notches or indentations (Figure 109) (44).

Since it has been reported that only protein fibers produce diamond-shaped stress diagrams or figures when subjected to the action of sulfuric acid, this simple and quick test can be used to advantage to distinguish among soybean, Aralac, Lanital, and all smooth protein fibers. These diamond-shaped stress figures are formed when 50% sulfuric acid is allowed to flow around the fibers on a glass slide under a cover glass. The diamonds appear at various points along the protein base fibers and are spaced irregularly along the length of the fiber. The diamonds can be seen for a short time and then they begin to

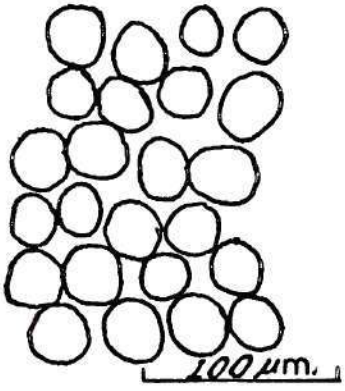


Figure 109. Aralac X200

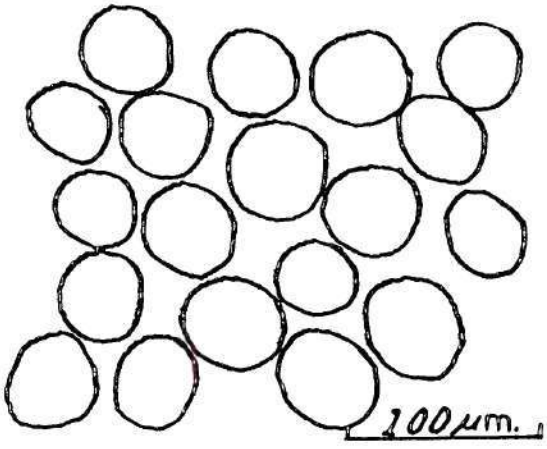


Figure 110. Soybean Fibers X200

change form and finally disappear as the fibers become wholly soft. The strain is released quickest in soybean fiber, slower in Lanital, and even more slowly in Aralac. An experienced microscopist is able to distinguish among the three fibers by varying the concentration of the sulfuric acid and noting the time required by each type of fiber to form the characteristic diamond stress patterns.

Another method of detection is found in the use of ultraviolet light. All samples must be extracted first with ether and then with alcohol to remove spinning or finishing oils and softening soaps. Undyed Aralac, Lanital, and wool have a natural fluorescence when examined under an H-3 mercury vapor lamp, using a Corning Violet Ultra No. 586 filter.

Fluorescence of Aralac, Lanital, and Wool

Fiber	Color of Fluorescence
Aralac	Bright, bluish white
Lanital	Dull, much yellower than Aralac or wool
Wool	Bright, white, yellower than Aralac

Soybean

Soybean fiber, such as Silkool in Japan and Soylan in the U. S., has been discontinued (47).

Under the microscope, soybean fibers appear very similar to the casein fibers previously discussed. The high circularity of the soybean fiber makes accurate diameter determinations easy by the width and cross section method (Figure 110). In all width measurements glycerin should be used as an embedding medium to avoid any swelling (13).

Peanut

According to Moncrieff (47), the only peanut fiber made commercially is Fibrolane C in England.

Zein

Zein is an odorless, nontoxic protein derived from corn. It is a by-product of corn processing, and it is made into a vegetable protein fiber, primarily used in blends. Apparently the zein fiber, Vicara, is no longer produced commercially in the U. S. (47).

Vicara is produced as an extremely soft, uniform fiber, light golden in color, being manufactured either bleached or unbleached. Under the microscope, it appears as a cylindrical, translucent rod with a nearly circular cross section. The specific gravity of the fiber is 1.25. It has a slight birefringence (0.004) which is not exhibited by the casein, soybean, or peanut fibers (3). Unlike animal and all other protein fibers, it is not drastically affected by alkalies.

Polyamide Fibers

Nylon is the generic term for any long-chain synthetic polymeric amide which has recurring amide groups as an integral part of the main polymer chain, and which is capable of being formed into filaments in which the structural elements are oriented in the direction of the axis.

The longitudinal view of nylon presents the filaments as very even in width over the entire length, smooth and lustrous as a glass rod, showing no surface structure at all.

The dull filaments show the same characteristics with the exception that a delusterant (titanium dioxide) is present. The cross sections of lustrous and dull nylon fibers are circular and extremely uniform in diameter (Figure 111). The contours are smooth and not indented as in viscose and acetate fibers. The dispersion of the pigment in the dull nylon filaments is similar to that of a medium dull rayon. Multilobed nylon is now produced (47).

The following materials will dissolve nylon yarn at room temperature: concentrated formic acid; phenolic compounds such as phenol, cresols, xylenols, and chlorinated phenols; and a saturated solution of calcium chloride in methanol. Nylon yarn, like other textile fibers, is subject to some slight deterioration when treated with bleaches of the ordinary types (3, 13, 44).

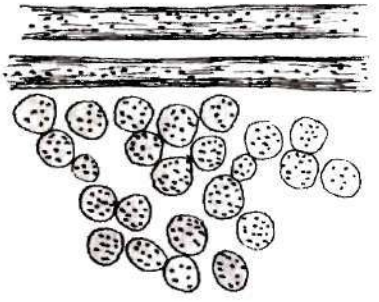


Figure 111. Nylon X200

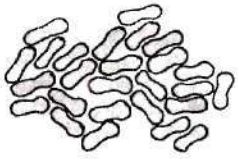


Figure 112. Vinyon X200

Polyvinyl Fibers

Probably the best known of the polyvinyl fibers are Vinyon, Saran and Velon (3).

When viewed under the microscope longitudinally, the filaments of Vinyon in their natural form resemble mercerized cotton with what appears to be a lumenlike channel running through the middle of the filament with an occasional twist. This illusion of a lumen is explained by the peculiar cross-sectional shape (Figure 112). At 500 diameters the two thick ends cast shadow in the longitudinal view with oblique illumination.

When Vinyon filaments are examined in organic solvents, such as bromonaphthalene, the fibers gradually dissolve by disintegrating first into splinters, which, as they diminish more and more, start to undulate.

In addition to the polyvinyl fibers mentioned, Dynel, a fiber which is a mixture of vinyl chloride and acrylonitrile, is being produced. The filaments show an irregular, ribbon-shaped cross section (44).

Polyacrylic Fibers

In this classification are synthetic fibers such as Orlon, Acrilan, and Creslan (47).

Orlon

The general appearance of this fiber is similar to Vinyon, but the surface of Orlon is irregularly striated to varying degrees. The shape of the majority of the cross sections is dumbbell or dogbone like that of Vinyon. The specific gravity is 1.17 compared with 1.14 for nylon. Orlon will melt and burn, leaving a hard, beadlike ash similar to that of cellulose acetate (3).

A chemical separation for Orlon, nylon, and acetate follows: Orlon is not affected by acetone or 88% formic acid, nylon dissolves in 88% formic acid, and acetate dissolves in acetone.

Acrilan

In cross section, the acrilan fiber is kidney-shaped or reniform (3).

Polyester Fibers

Dacron

Dacron fiber is manufactured from a chemical composition of ethylene glycol and terephthalic acid. The microscopic properties are similar to those of nylon (3). Terylene, a fiber produced in England, is similar to dacron.

Polyethylene or Polythene Fibers

Reevon and Wynene are examples of polyethylene or polythene fibers. They are round or elliptical in cross section (3).

Note: New man-made fibers are introduced to the market and old ones discontinued, so that trade journals and other up-to-date references should be consulted by those especially interested.

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CHAPTER VII

THE MICROSTRUCTURE OF THE PLANT CELL WALL

The cell wall of various plant cells, particularly the "fiber," is the basic fibrous raw material of the papermaker. In view of the many processing treatments to which these fibers may be subjected by the papermaker it is important to understand the basic structure of the papermaking fibers. In the previous chapter, some of the morphological features of aid in identification were described. For a more thorough understanding it is necessary to discuss the microscopic and submicroscopic architecture of the plant cell wall.

The importance of this subject is evidenced by the fact that hundreds of researches on the chemical and physical nature of papermaking and textile fibers have been pursued in the past three or four decades. It is, of course, unfeasible to consider more than a small number of these. Rather, the modern concept of the detailed cell wall structure of the wood "fiber" as well as the cotton seed hair and some of the more commonly encountered nonwoody fibers used in the paper industry will be summarized. Several authoritative reviews are available for the student who requires additional information on this subject (1-8, 90-92).

THE WOODY PLANT CELL

The fibrous cells of wood (the tracheid in softwoods and the fiber in hardwoods) are the principal source of fibrous raw material in the North American paper industry. There has been considerable research in this field since Ritter reopened the study of the chemical nature of the woody cell wall in 1925 (9-11). Prior to this, there had been a few pioneering efforts [see (12)], but for the most part great reliance was placed on microscopic stains to determine the chemical nature of cell walls. The use of stains for this purpose is satisfactory only if it is supported by chemical studies under the microscope but otherwise it may lead to erroneous conclusions (13).

TERMINOLOGY

In 1934, Kerr and Bailey (12) recommended that the following terminology be used in discussing cell wall researches. It was recognized that future changes might be necessary but it was evident that a common denominator was needed to prevent further confusion. The essential elements of their suggestion are shown in Fig. 113.

Between the walls of two adjacent cells is a layer, which is considered as a cementing substance common to neither, known as the middle lamella. Proceeding from this separating layer into the cell wall toward the lumen of the cell the primary wall is first encountered. Interior to the primary wall is the secondary wall which may consist of several layers. In order to prevent confusion with earlier usage, Kerr and Bailey suggested [in agreement with van Iterson (14)] that the old middle lamella, actually composed of the true middle lamella, or intercellular substance, as well as the primary walls of contiguous cells, be known as the compound middle lamella.

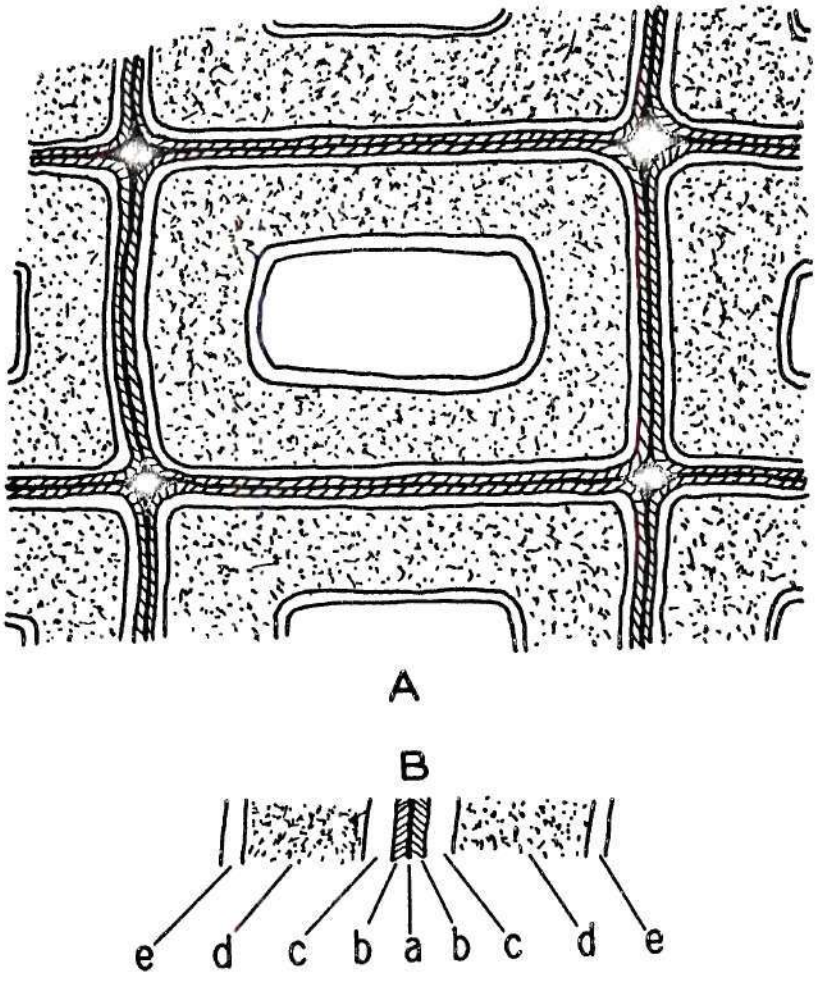

Figure 113. A. Diagrammatic Transverse Section of One Entire Coniferous Tracheid and of Parts of Eight Others. B. Section of Adjacent Walls More Highly Magnified; (a) Truly Isotropic Intercellular Substance, (b) Cambial or Primary Wall, (c) Outer Layer of Secondary Wall, (d) Central Layer of Secondary Wall, (e) Inner Layer of Secondary Wall

Fortunately, since this suggestion was made, most workers have used this terminology and only a few (15-18) have insisted on using other terms, especially in connection with parts of the secondary wall. These few men doubtless have felt that certain important connotations were thereby derived. In the subsequent discussion of the chemical and physical nature of these various parts of the cell wall an attempt will be made to correlate these terms.

MIDDLE LAMELLA

The true middle lamella is located between the cambial or primary walls of adjacent cells. It is also known as intercellular substance and is often referred to as cementing substance by many authors. In the region of newly formed xylem cells, the middle lamella contains pectic polyuronides but within a short period of time the formation of lignin masks the original material so that in mature woody tissues the intercellular substance has a very high

percentage of lignin and is isotropic (12). Ritter found that 70% of the lignin of wood occurred in the compound middle lamella (9). Recently, however, Berlyn and Mark (93) suggested that about 40% agreed more closely with the existing data. The compound middle lamella microdissected from Douglas-fir sections by A. J. Bailey was found to contain 71% lignin and 14% pentosans (19).

Lange studied the lignin directly in the cell wall with microspectrographic methods in the ultraviolet range and found a heavy concentration in the compound middle lamella, about 73% as an average value for spruce (20). Lange and Kjaer have used the interference microscope recently to study this distribution (21). According to Lange (22), the compound middle lamella has a more porous structure than the secondary wall, which facilitates diffusion and penetration processes in the former. Occasionally it appears that there is direct contact of adjacent primary walls over small areas and that no intercellular substance is present at such points (23).

Maceration Techniques

At this point it may be of interest to mention various methods which have been used to macerate small pieces of wood so that the fibers might be isolated for morphological study. Schulze's (24) classic method involving nitric acid and potassium chlorate, or modifications of it, is still in use. The method of Spearin and Isenberg (25), using sodium chlorite and acetic acid, is a more gentle one, which normally does not degrade the fibers drastically, and is relatively easy to use. Franklin (94) has devised a technique using acetic acid and hydrogen peroxide. Wilson has recently presented a resume of various maceration techniques (26). Recently, Burkart (95) proposed a method in which the wood is treated with triethylene glycol containing an organic catalyst, such as phenolsulfonic acid or p-toluenesulfonic acid, at 130°C.

THE PRIMARY WALL

The primary wall, designated as P or 1°, is the outermost layer of the woody plant cell (Figure 113 and 114). It is often referred to as the cambial wall because it is formed during the cell division of the cambium. Thus, in the ontogeny of the woody plant cell, the primary wall is formed from the protoplasm in the phase of origin and increases in area in the phase of enlargement, apparently in accord with the intussusception theory, in which new units of wall material are intercalated between old units which have separated. One of the most important features of the primary wall is that its surface growth is not accompanied by a marked increase in thickness. The primary wall is quite thin, usually less than 0.2 μm. in thickness.

Basically, the cambial wall is composed of cellulose, noncelluloses, and water. It should be kept in mind that the cellulose forms, on the average, but 9% of the volume of the fresh primary wall, and only 30% of the dried one. By the removal of the noncellulose and the water, the wall therefore shrinks considerably, not only in surface, but to an even greater extent in thickness. The submicroscopic units of the cell wall, the microfibrils, are, hence, much more compact than they are in the original condition. The microfibril is a discrete threadlike structure of 30-300 A. thickness and indefinite length (96). The cross section appears to be somewhat flattened. The fibril, of which the microscopist has long spoken, is a microscopically visible structure composed of bundles of microfibrils.

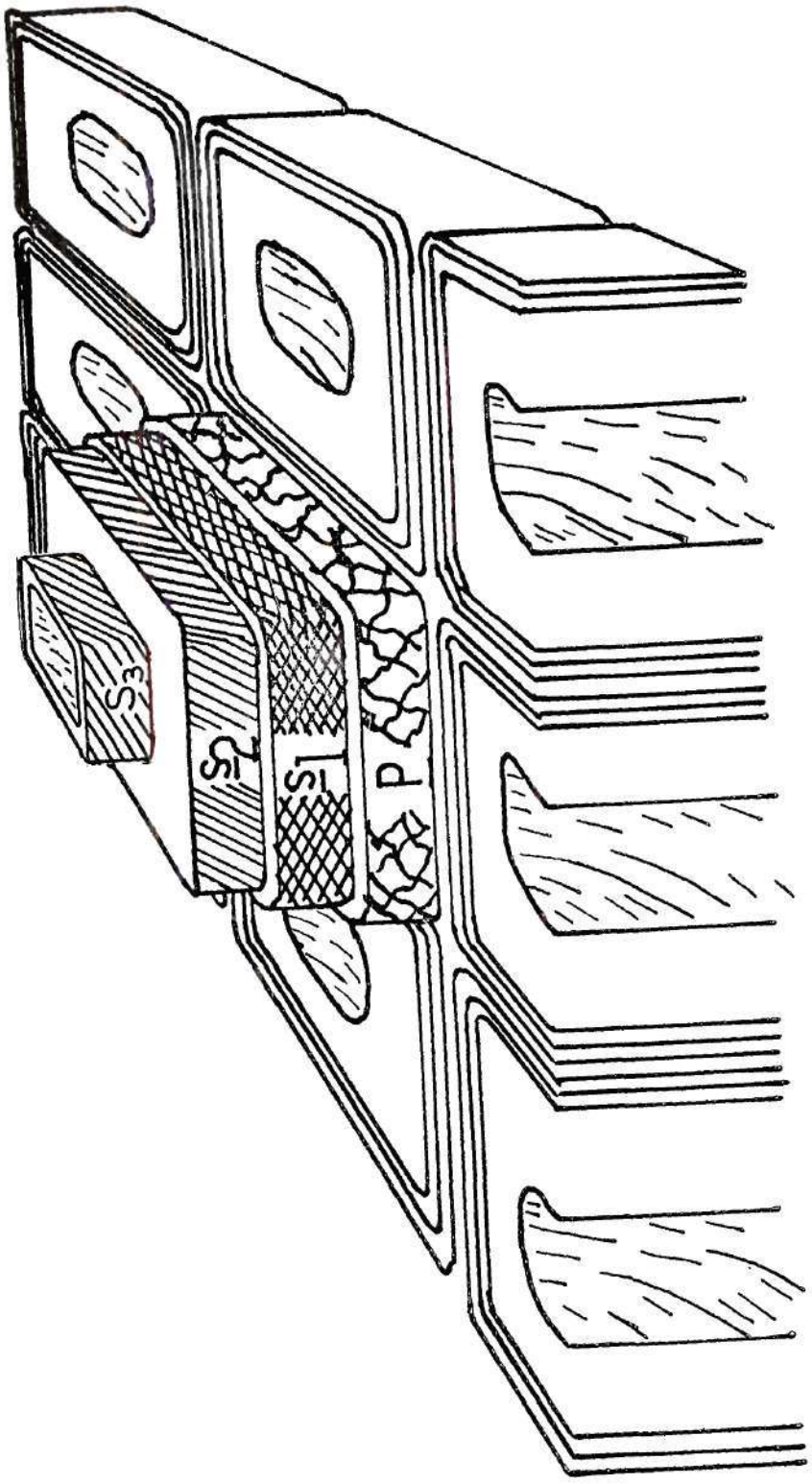

Figure 114. Schematic Drawing, Showing Microfibril Orientation Common in Many Coniferous Species

The microfibrils ultimately are composed of cellulose molecules arranged in crystalline or amorphous form. Although there are several reasons for assuming that the cellulose is not crystalline throughout, but that there is also semi-crystalline and amorphous cellulose, a discussion of this is beyond the scope of this book. It is not known how and where cellulose molecules are synthesized by the protoplasm, and how and where the microfibrils arise from the molecules.

Positive birefringence in the cell wall of a higher plant is very strong indication of the presence of cellulose. The birefringence of the cellulose is due to several causes; there is crystalline birefringence of the crystallites in the microfibrils, molecular birefringence in the more or less amorphous parts of the latter, and rodlet birefringence in the aggregate formed by the microfibrils and the interfibrillar material (see Chapter III for a discussion of polarized light).

The optical properties of the primary wall prove that the main part of the cellulose micelles must be oriented either transversely, or else in a helix with low pitch. This conclusion has been fully confirmed by the results of x-ray analysis and of electron microscopy. Preston and Wardrop (27) have studied the structure of the primary wall of cambium cells by means of the x-ray method, using strips of cambium obtained from Scotch pine. The data are consistent with a transverse orientation.

In addition to the cellulose, the primary wall presumably contains pectic polyuronides, and possibly cellulosans (6, 12, 28). As the cell matures, this wall is infiltrated with lignin, so much so that until the careful studies of Kerr and Bailey (12) demonstrated that the cambial wall was anisotropic and basically cellulosic, the lignin of the primary wall and that of the intercellular substance masked the true nature of the layer and it was undetected. In other words, the middle lamella of the older workers actually included the primary walls of adjacent cells.

At this point it may be of interest to examine the fiber after it has been separated from its neighbors in chemical pulping. Carpenter and Lewis (29) have shown very definitely the existence of a lignin sheath encasing very raw jack pine kraft fibers. Ritter (30) has depicted a winding layer removed from a delignified spruce fiber. Apparently, these more or less definite spirals may be closely or widely spaced. Bixler (31) demonstrated that pulp fibers are always encased by the cambial wall. According to Bucher (16, 32) it is possible to loosen an exterior layer from pulp fibers by refining. The closing membrane of the pits are still attached to this layer. One can conclude, therefore, that this is the primary wall or a combination of the primary and secondary walls. Schramek and Stenzel (33) and Klauditz and Stegmann (34) found that the action of swelling and cooking agents often loosened a ribbonlike layer.

Kallmes (35) found that unbleached spruce sulfite fibers had considerable lignin remaining in the outer layers (P and S_1). It appears that these layers are the last part of the cell wall to lose their lignin in pulping.

Even in the very earliest cell wall studies the phenomenon of "balloon swelling" was observed when the isolated fibers were treated with swelling agents such as acids and alkalies. Hundreds of investigators have observed this swelling effect on numerous fibers. Cuprammonium hydroxide (Cuam) has been used commonly and cupriethylenediamine (Cuene) is a very good agent (Figure 115). The balloons or beads appear chiefly in concentrated solutions when the inner layers of the cell wall burst through the outer layers at weak points.

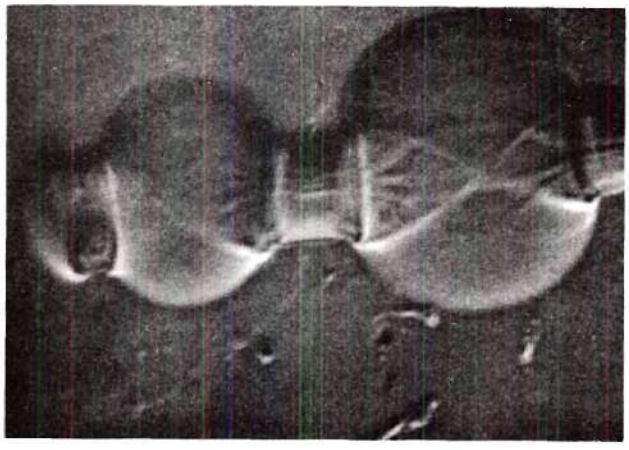

Figure 115. Spruce Sulfite Fiber Swollen in Cuene [X800 (37)]

There has been disagreement as to whether the primary wall or the outer layer of the secondary wall causes the constrictions resulting in balloon formation. Wardrop (36) has presented a good discussion of this phenomenon in which he emphasizes the fact that in coniferous tracheids it is the outer layer of the secondary wall. One of his illustrations showed fragments of the primary wall on the surface of the fiber. Isenberg and Smith (37) in their cinéphotomicrographic studies of spruce pulp fibers detected fragments of primary wall even on fibers which had been bleached and beaten. In 1943, Clark (38) used the British disintegrator to rub off the primary wall of bleached western hemlock.

Observation of the surface of a macerated vessel member showed it to have a netlike structure (97, 98). Procumbent ray cells also exhibit a netlike structure in the primary wall (97).

THE SECONDARY WALL

As previously mentioned, the terminology suggested by Kerr and Bailey (12) will be used to describe the various layers which occur in the secondary wall of the woody plant cell. The secondary wall is usually considered to be a series of layers, characteristically grouped into three major types, although Bailey and Kerr (39, 40) have demonstrated a radial orientation in some species. Occasionally,

there is a combination of both concentric and radial orientation (Figure 116A). The three layers of the secondary wall are designated as the outer (S_1), the central (S_2), and the inner (S_3). Conflicting terms used by other investigators will be discussed later.

The secondary wall appears to be two interpenetrating systems, one of cellulose and other polysaccharides, and one of lignin. Lange (20, 22) has studied the distribution of chemical components across the cell wall using an ultraviolet absorption technique. He found a concentration of lignin in the compound middle lamella with a diminution toward the cell lumen. The polysaccharides were in greatest proportion next to the lumen. Cellulose is the dominating component around the lumen and the amount in the outermost layer is only about half of that around the lumen. Consequently, there is more space in the outer regions for deposition of hemicellulose and lignin. Since it is impossible to study the carbohydrates in the cell wall by microspectrographic methods in the presence of lignin, Lange (41) has also examined cross sections of fibers from laboratory and commercial pulps. Kallmes (35) found in unbleached spruce sulfite fibers that the alpha-cellulose and hemicelluloses were slightly higher and crystallinity was greater in the central layer of the secondary wall as compared with the P and S_1 fraction.

Ritter (10, 11, 30) and Lüdtke (42), by means of various swelling agents, have separated the secondary wall into elongated fragments, termed fibrils. Lüdtke, furthermore, has postulated that "cross elements" and "skin substance" surround these fibrils but his hypothesis has not gained wide acceptance and it has been pointed out that it is unnecessary to have "skin substance" in order to have ballooning in fibers (36).

Outer Layer

The outer layer (S_1) of the secondary wall shows an orientation of microfibrils at considerable angle to the fiber axis, about 40-55°. Although this layer is generally quite thin, it has been shown, in some cases at least, by Wardrop (43), Emerton (44-46), and Meier (18), to consist of two lamellae whose microfibrils are inclined at the same angle in opposite directions so that a conspicuous crossed structure is observed (Figures 114 and 117). Wardrop (43) believes it is composed of four lamellae arranged in two pairs differing in the thickness of the fibrillar bundles. But often one of the two directions prevails. The thickness of the whole layer is 0.1-0.2 μm. Wardrop also observed that in drying of S_1 fragments the shrinking appears to be confined to the axial direction.

The structure of the S_1 layer evidently shows a greater resemblance to that of the primary wall, which may also consist of lamellae and in which more or less similar crossed structures have been observed than to the S_2 layer in which such structures have never been found. This resemblance, plus the fact that the lignin content of the S_1 layer is relatively high, has led some workers (18) to think of the S_1 layer as a transition layer (Übergangslamelle).

The question as to which part of the woody cell wall was responsible for the constrictions noted in typical "balloon swelling" of fibers has evoked considerable discussion throughout the years. The consensus is that the S_1 layer is chiefly responsible (36, 47) and that the swelling of the S_2 layer causes breakthrough at weak spots in the wall such as slip planes, pits, and other discontinuities. The

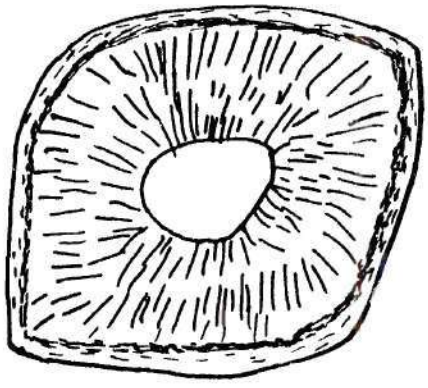

Figure 116A. Radioconcentric Layering in S_2 Layer

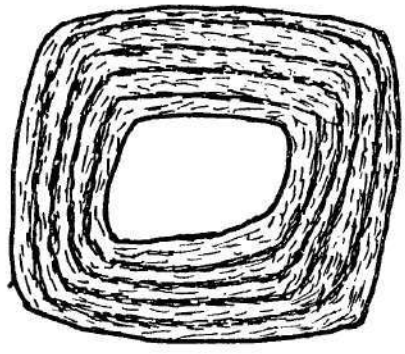

Figure 116B. Concentric Layering in S_2 Layer

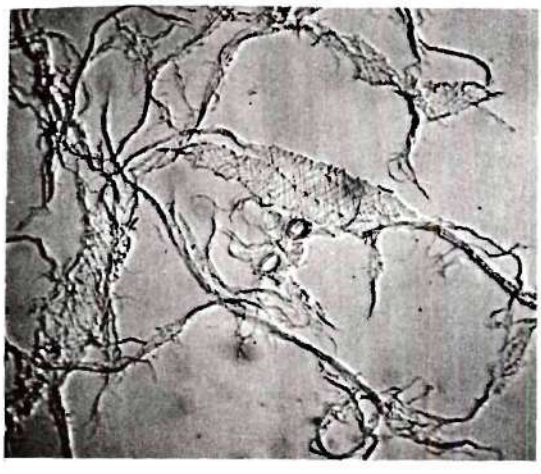

Figure 117. Spruce Sulfite S_1 Layer, Showing Two Lamellae [X400 (35)]

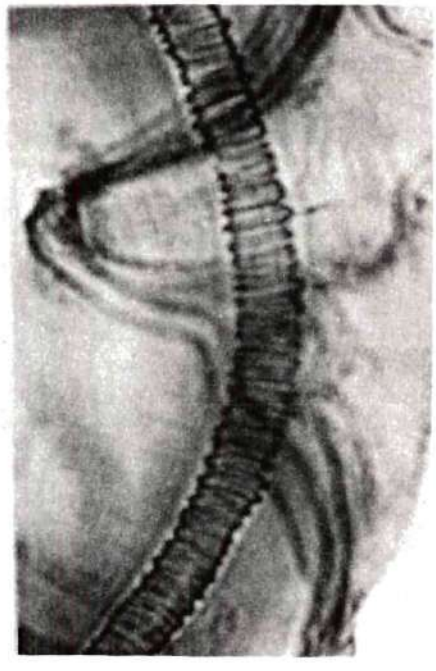

Figure 118. S_3 Layer in Scotch Pine [X250 (17)]

inability to produce ballooning, however, does not prove the absence of layers with the transverse orientation of the cellulose. The swelling may be insufficient, either because the cell wall is too thin or because it contains too much lignin or hemicellulose.

Central Layer

It may be said that the central layer of the secondary wall is the part that characterizes a particular cell wall. Whereas the thickness of the primary wall, the outer (S_1), or the inner (S_3) layers show little variation in the various fibers, the thickness of the central (S_2) layer may show appreciable differences and, hence, govern the total cell wall thickness. A common example of such variability appears in the walls of springwood and summerwood coniferous tracheids of the same growth ring.

The central layer of the secondary wall exhibits an orientation of fibrils at a rather small angle with the fiber axis. It is worthwhile to draw attention to the fact that the S_2 layer consists nearly always of thinner layers with identical or only slightly different orientation (Figure 116B). These lamellae and the interlamellar layers obviously differ in chemical constitution (39). Although nothing is known of the nature of these differences, it seems likely that layers containing much cellulose and little hemicellulose and lignin, alternate with layers containing little cellulose and much hemicellulose and lignin. The perfectly parallel orientation, the very dense structure, and the arrangement of the microfibrils in bundles, are characteristic features of the cellulose in the secondary wall. In higher plants, until now, not a single instance has been found of a cell wall whose secondary thickening (the third phase in the ontogeny of the woody plant cell), at least in so far as the cellulose fraction is concerned, is not due to apposition. However, an increase in thickness by intussusception of noncellulose, such as the deposition of lignin between the existing constituents of the cell wall, is not uncommon.

Inner Layer

The inner layer of the secondary wall has been studied intensively in recent years by Bucher (17, 48) and Meier (49). With some rare exceptions, normal tracheids and fibers possess an S_3 layer with a rather flat spiral structure and with a different chemical composition (Figure 118). One other observation made by Bucher, who used Victoria Blue, an amphoteric dyestuff, in his studies, deserves special mention, namely, that the S_3 layer extends in the bordered pits of the tracheids over the inside of the overhanging margin, where it is, therefore, in contact with the primary wall. Bucher (17), Meier (49), as well as Lüdtke (15) and other investigators, have elected to refer to the inner layer of the cell wall as the tertiary wall.

Several authors (50-52) have reported the presence of warty protuberances from the inner layer toward the cell cavity, or lumen, of many species of wood. This structure appears to consist of "warts" between and over which, a layer of amorphous material often exists. Even though it develops during the death of the protoplasm, it is to be regarded as a real part of the cell wall. In most cases the size of the "warts" is of submicroscopical dimensions, but many species also have larger ones visible with the light microscope. The appearance and

distribution of the "warts" can vary greatly among genera, species, individuals, and even within a particular fiber (99).

In certain species (e.g., Douglas-fir), whose fibers have spiral thickening next to the lumen, it has been determined that these thickenings are also composed of microfibrils, and on their surfaces the microfibrils have an interwoven structure similar to the S_3 layer.

Bordered Pits

Liese (53, 54, 100) and others (52, 55-57) have studied the detailed structure of the torus and closing membrane of bordered pit-pairs. In the softwoods the torus is microfibrillar in nature. The perforated membrane which surrounds the torus is made up of bundles of microfibrils arranged in a preferential radial pattern from the central thickening outwards. The openings in the membrane vary in size but there are often spaces of 0.1-0.2 μm., permitting water to move freely. By means of these bundles the torus is suspended in the middle of the membrane.

In hardwoods the pit membrane resembles the primary wall in microfibril organization but is frequently incrusted. No openings, resolvable with the electron microscope, have been found. The same type of membrane exists in vessel members, tracheids, and fibers. Water movement between cells separated by such membranes occurs only through the mechanism of diffusion (101).

Reaction Wood

Australian investigators (58) have studied the orientation of the microfibrils in the cell wall layers of the fibers of reaction wood. In the compression wood tracheids, Wardrop and Dadswell (59) reported that the S_3 layer was missing from the cell wall. In the outer layer the micelles were inclined at a large angle and in the S_2 layer at a relatively smaller angle. Longitudinal striations are visible in this inner wall (S_2). These investigators (60) found, however, that in tension wood fibers several conditions may exist. All three layers of secondary wall may be present, but S_3 and even both S_3 and S_2 may also be absent. On the inside almost always a remarkably thick layer of an aberrant character, the so-called gelatinous layer, occurs (102).

THE COTTON SEED HAIR

The cell wall structure of the cotton seed hair is better known than that of any other vegetable fiber. Although it has a very peculiar structure which cannot be regarded as characteristic of plant hairs, its importance in the paper industry justifies a rather detailed discussion of it.

A century ago Naegeli noted that the cell wall of cotton seed hair demonstrated "ballooning" after treatment with sulfuric acid and that in the swollen parts two sets of crossing striations could be detected. The lamellar structure of the secondary wall was also known to him [see (1), p. 228]. In 1919, Balls (61) showed that the lamellae of the secondary wall are probably daily growth rings. Kerr (62), using very thin sections which were first swollen and then

stained with Congo red, could show that each of the growth rings consists of two lamellae, a denser one which stains more strongly, and a less dense one which does not stain so strongly. Together they are about 0.35 μm. thick. According to Hock, Ramsay, and Harris (63) the later rings are thinner than the earlier ones. There are some 25 to 50 of these concentric layers of cellulose each of which is a sheet of microfibrils tightly packed in parallel alignment. Individual microfibrils are strap-shaped and grade down to 100 A. in width and about 30 A. in thickness. The length appears to be indeterminate but they are at least 5 μm. long. In each layer, the microfibrils are arranged in a spiral fashion with respect to the main axis of the fiber, such that the average angle of the helix is about 30°, ranging, as a rule, between 20 to 30°, but may increase to 45°. At the reversals, which occur at the twists or convolutions, the angle decreases, of course, to 0°.

PRIMARY WALL

The outer layer of the fiber is called the "primary wall" because it is the first membrane produced in the formation of the fiber on the surface of the seed. It is composed of cellulose microfibrils also, but they are not arranged in parallel fashion. In the primary wall the cellulose microfibrils constitute a skeletal network which is incrusted with noncellulosic materials of pectinaceous, proteinaceous, and waxy nature. Its noncellulosic content is affected very little by mercerization, but the primary wall is purified to a considerable extent by the normal textile processes of scouring and bleaching.

The primary wall, especially after the removal of the noncellulose, is exceedingly thin. On the average, but 9% of the volume of the fresh wall, and only 30% of the dried one is cellulose. The wall, therefore, shrinks considerably, not only in surface, but to an even greater extent in thickness by the removal of the noncellulose and of the water, and the texture formed by the microfibrils in the preparations for electron microscopy are much more compact than it is in the original condition. Kerr (64) was able to show that in fresh hairs the primary wall may easily be shifted over the secondary wall by scraping.

The structure of the primary wall in hairs has been studied by Kling and Mahl (65, 66), Roelofsen and Houwink (67-69), Tripp, Moore and Rollins (70, 71), and O'Kelley and Carr (72). The results are in good agreement. On the inner side of the wall a dense mat of microfibrils with a predominantly transverse orientation is noted. The microfibrils are not straight but wavy, and they form bundles that are interwoven. On the outer side the fibrillar structure is much less dense, and the fibrils and fibril bundles moreover are axially oriented. The primary wall retains its structure in mature hairs (65, 66, 71). Jurbergs (103) states that cotton linters have essentially the same surface structure as staple fibers.

WINDING LAYER

Just beneath the primary wall is a layer known as the "first layer of secondary thickening" or the "winding layer." It consists of cellulose microfibrils arranged in alternating bands of open network and of dense sheets of closely packed microfibrils. As far as tests with microstains indicate, there is no noncellulosic material in the winding layer. Neither the primary wall nor the winding layer is thicker than 0.1-0.2 μm., and together they ordinarily do not constitute more than 5% of the fiber by weight, but, because of their location at the periphery

of the fiber and because of the nature of their physical organization, they influence the physical behavior of the fiber. Anderson and Kerr (73) showed that the outermost set of striations is confined to the first day growth ring. According to them the spiral usually, but not invariably, differs from that in the rest of the wall.

Hock, Ramsay, and Harris (63) emphasized the fact that an S-spiral in the S_1 layer is always accompanied by a Z-spiral in the next lamella and presumably in succeeding ones. The S_1 layer, or winding layer, has a coarser fibrillar structure, and the spiral has, as a rule, a somewhat lower pitch. The coarse helical structure of the S_1 layer could be demonstrated very conspicuously by Rollins (74) in hairs that were treated with nitrogen dioxide. The wall of such hairs never shows ballooning, but the outermost layer is ruptured in the form of a spiral.

There are indications of the presence of an S_3 layer in the appearance of striations with a larger angle (30-45°) than that of which undoubtedly belong to the S_2 layer (75).

OTHER NONWOODY FIBERS

BAST FIBERS

Krabbe (76), after a study of the phloem fibers of various Apocynaceae, Asclepiadaceae, Linum, Urtica, etc., concluded that the wall always consists of two and sometimes of three layers with an alternating helical orientation of the cellulose. The outer layer may show an S- or a Z-striation.* Furthermore, it was noted that the second layer is often rather thick, and that it then consists of several thinner layers, which were called lamellae because the spirals are oriented in the same direction, although not rarely with a different pitch. Naegeli (77) had previously observed several lamellae in hemp fibers which showed ballooning. Hock (78), in 1942, found that lamellae were recognizable in flax fiber after removal of the cellulose and staining with ruthenium red.

Roelofsen (79) detected three layers in flax and hemp fibers. The S_1 layer is always present and well-developed in hemp fiber, whereas in flax fiber it is either very thin or, at least in retted flax, completely absent. On the other hand, the S_3 layer is well-developed in flax, whereas it may apparently be lacking sometimes in hemp.

The direction in which these fibers twist under the influence of swelling and shrinking agents is in agreement with their structure. The reason the twist of the hemp fiber differs from that of flax and also ramie fibers, is apparently because the S_1 layer is mechanically of far greater importance in the hemp fiber.

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*When a Z-helix is placed vertical, i.e., in the direction N-S under the microscope, the part nearest to the observer is parallel to the middle part of the letter Z. The mirror image of such a helix is an S-helix [see (1), p. 105].

The direction of the twist is constant for each species, and apparently even for each family of plants (80, 81). That isolated flax and hemp fibers may be distinguished by means of their optical behavior has long been known. Ramie fiber shows similar optical properties as flax although it has greater thickness than both flax and hemp.

According to Preston (82) the greater part of the lignin is deposited in the external layer of the jute fiber almost doubling the thickness of the layer. In all phloem fibers with little or no lignification, many nearly transverse markings, called dislocations or slip planes, are present.

SEED HAIR

In milkweed floss, Rollins (83) observed that the inner layer of the wall has a different chemical composition than the remainder. Roelofsen and Houwink (68) reported the microfibrils in the outer surface of the primary wall of the seed hairs of Ceiba and Asclepias to be oriented longitudinally and in the inner surface transversely.

MONOCOTYLEDONS

The greater part of our knowledge with regard to the helical structure and the stratification of these fibers as revealed by the aid of the ordinary microscope is due to Sonntag (84). In the species he studied the S_1 layer always has an S-helix and the S_2 layer a Z-helix, and the slope of the first is always more gradual than that of the second. A painstaking study by Preston and Middlebrook (85) has confirmed the conclusions of Sonntag for sisal fibers. The twist test of Newman and Riddell (81) also indicates a predominant Z-structure.

Various investigators have found more than three layers in several monocotyledonous fibers, e.g., Pandanus odoratissimus (39, 86). Others found this condition in various kinds of bamboo (87, 88) and in sugar cane (89). A primary wall and a three-layered secondary wall are evident in rye straw fibers (104).

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CHAPTER VIII

FIBER ANALYSIS

INTRODUCTION

It is not intended to present a detailed historical review of the development of fiber analysis; however, it does seem fitting to mention those pioneers of the second half of the 19th century who contributed so much to this development.

The foundations of fiber histology were laid by Quekett in "Lectures on Histology" (1851), and were first applied specifically to the examination of paper by Suffolk, who described the results in "Microscopical Manipulation" (1869), a series of lectures delivered before the Quekett Club. The scientific study of the microscopical examination of fibers was continued by Wiesner (1867) and Vétillart (1876). The technology of paper microscopy was specially developed in Germany by Herzberg (1888), Behrens (1896), and Hanausek (1901), and in Austria by Höhnel (1888).

At the time of the founding of the Paper Section of the National Bureau of Standards in 1911 and the organization of the Technical Association of the Pulp and Paper Industry in 1915, standard methods of procedure for fiber analysis were employed to some extent.

Paper mill chemists in Austria, Canada, France, Germany, Great Britain, and the United States began to seek for methods of differentiating the various kinds of papermaking materials. Publication in technical journals and comparison of results led to tentative and standard methods of analysis. The paper industry was no longer satisfied to know if the furnish consisted of rag, groundwood, chemical wood pulps, straw, or bast fibers; it also wanted to know whether the chemical pulp was soda, sulfite, or sulfate, and whether it was unbleached or bleached. In addition, a fairly accurate determination of the relative amounts of the various kinds of fibers was required. Many investigators were trying constantly to develop more suitable staining differentiations and methods for quantitative determinations.

The problem of fiber analysis became even more complicated when new methods of cooking and bleaching, which produced variations in the stain reactions of the different pulps, were adopted by the industry. Likewise, the large number of variations in the older processes has increased the need for more exact qualitative and quantitative determinations of paper furnishes. It seems desirable, therefore, to review the various methods of analysis and the steps of these methods to determine their usefulness for present-day fiber analysis.

SAMPLING

TAPPI Revised Tentative Standard T 401 m-60 states that the test specimen shall consist of approximately 0.2 g. of paper or paperboard torn from different portions of the test sample so as to be representative of it. With many papers this weight of sample usually involves about 25 cm.^{2}, or 4 in.^{2}. In corrugated container board, or laminated sheets such as multicylinder board, it may be

desirable to separate the components and analyze them individually. For these samples, then, it may be appropriate to use 0.2 g. of each layer.

While this is the recommended amount of specimen, there are times, of course, when this much sample is not available. In an earlier version of this standard (1942) it was suggested that an area of 3 cm.^{2} (1/2 in.^{2}) would be recognized for quantitative analysis and 0.9 cm.^{2} (1/8 in.^{2}) for a qualitative determination. It is important to note if less than the recommended amount is used in an analysis.

DISINTEGRATION OF SAMPLE

The TAPPI standard method of disintegration, the C. G. Bright method (1), and the method developed at The Institute of Paper Chemistry (2) are more or less similar and consist of the following steps:

The sample is torn into small pieces, placed in a small beaker, covered with 1/2% sodium hydroxide, then brought to a boil on a hot plate; the alkaline solution is decanted, and the sample is washed twice with distilled water, covered with 0.05N hydrochloric acid, and allowed to stand several minutes; the acid is decanted and the sample is again washed several times with distilled water. After the water is drained off, the pieces of paper are rolled into small pellets between the fingers, and placed into a 500-ml. Erlenmeyer flask; 250 ml. of distilled water are added and the contents are shaken vigorously until the fibers are thoroughly disintegrated. The suspension is poured back and forth from a 250-ml. graduate several times. Finally, 125 ml. are measured out and diluted to 250 ml. After shaking thoroughly, 125 ml. of the mixture is diluted to a suspension having a consistency of about 0.05%, and then is poured into test tubes. However, because different fibers vary considerably, it is often preferable to prepare standard suspensions of approximately correct consistency for the different furnishes. Thus, suspensions from papers to be analyzed can be made up rapidly without weighing and measuring exactly. Of course, the bringing of these suspensions to their proper consistency becomes easier as one gains experience.

Any papers containing wool fibers, such as roofing papers, must not be so treated since the alkali may dissolve the wool.

TREATMENT OF SPECIAL PAPERS

Some papers must be given special treatment before they can be properly disintegrated. Others are so highly colored that they are not decolorized by the standard method of disintegration and the dyes must be removed before disintegration is effected. Standardized methods cannot be specified for the disintegration of papers containing tar, asphalt, rubber, viscose, etc., or parchment papers, because the procedure needs to be varied according to the material, the amount present, and the nature of the treatment. The following methods are suggested as guides.

Tar and Asphalt-Treated Papers

According to Herzberg (3), the sample is prepared by digesting it with heavy coal-tar oil for one hour on a steam bath, pressing it between blotters, then treating it with medium coal-tar oil on a steam bath, and again pressing it between blotters. It is then extracted with benzene until the solution drains clear from the thimble. Caustic soda should not be used in the disintegration of these papers, because of the possible presence of wool fibers.

According to a method developed at The Institute of Paper Chemistry (4), the sample of felt saturated with asphalt is extracted with trichlorethylene in a Soxhlet until the liquid siphons over clear (about 7 hr.), then air dried. The sample extracted in this manner is completely free from any tar and thus causes no difficulty for fiber analysis.

In one of the methods recommended in TAPPI tentative standard T 401 m-60, several 250-ml. beakers are half filled with carbon tetrachloride. The sample is cut into convenient squares and immersed in the first container. The squares should be moved about and the liquid agitated. After several minutes in the first container, the squares are transferred to the next container, using forceps. Do not allow the squares to dry. In the case of laminated papers, the sheets may be separated easily after the first or second soaking and this should be done, removing any scrim or mesh, which can then be treated separately, if desired. The sample is transferred into fresh solvent until the liquid remains clear after the sample has been agitated in it for several minutes, then the sample is removed and allowed to air dry. After drying, the sample is disintegrated in the usual manner.

Rubber-Treated Papers

Rubber-treated papers are disintegrated by allowing them to stand in chloroform for 24 hours, or extracting with anisole, and washing with benzene-alcohol (3). It is expedient to remove the large amount of rubber from artificial leather (another form of rubberized paper) by soaking it in turpentine from one day to a week, depending upon the thickness of the material.

Another method follows: The sample is dried over phosphorus pentoxide at room temperature for one hour at the highest vacuum attainable with a Hy-Vac pump. The sample should be cut into pieces about 1/4 in. square before drying. Two grams of the sample are accurately weighed and extracted with acetone in an A.S.T.M. rubber-extraction apparatus to remove resins, softeners, etc., from the sample which would otherwise be weighed and calculated as rubber. After the acetone extraction is complete (usually overnight), the sample is again dried in a vacuum and weighed to determine the amount of acetone-extractable material. It is then placed in the extraction flask and sufficient o-dichlorobenzene added to cover the sample. This is heated to approximately 120°C. for two hours, after which the liquid is poured off and replaced by fresh solvent and again heated for two hours. This treatment will, in most cases, remove all rubber. The sample is then placed in a siphon cup and extracted for two to four hours with chloroform to remove the last trace of o-dichlorobenzene. It may then be dried and weighed, or further extracted overnight with water, to remove water-soluble materials, if present. o-Nitroanisole appears to be more efficient in extracting vulcanized

rubber than o-dichlorobenzene, and may be used in place of the latter. Whichever solvent is used, care must be taken that the sample is not overheated with resultant dehydration of the cellulose. If the rubber in the sheet is known to be unvulcanized, xylene is a satisfactory solvent for the extraction (5).

A third method consists in extracting the sample for six hours in a Soxhlet with cumene (isopropylbenzene), drying and then boiling with water to which Tergitol has been added or, in very rare cases, with 1% sodium hydroxide. Usually cumene removes about 98% of the rubber (5).

Viscose-Treated Papers

Papers treated with viscose are difficult to disintegrate by the usual procedures used in fiber analysis. Applying Weber's method of disintegrating filter paper in hot calcium nitrate solution, it was found that papers containing viscose also rapidly disintegrated into individual fibers, by stirring with hot concentrated (50%) calcium nitrate solution for periods of about 1/2 hour or more, depending upon the amount of viscose present. The subsequent stain reactions on the fibers are not altered. The method has also been tested on wet-strength papers treated with synthetic resins, but was not found applicable (6).

Wet-Strength Papers

Papers treated with polyvinyl copolymer, polyvinyl chloride-acetate, cellulose acetate butyral, chlorinated rubber, polyvinyl butyral, or ethyl cellulose are first soaked in acetone for a length of time depending upon the amount of resin present and then given the regular sodium hydroxide treatment (7).

Papers treated with chlorinated rubber, ethyl cellulose, or melamine resins are first boiled in 5% aluminum sulfate solution for five to twenty minutes depending upon the amount of wet strength present, washed, and then given the usual boil in sodium hydroxide solution.

Because of the convenience of storing a crystalline compound, sulfamic acid has been suggested as an agent for hydrolyzing the wet-strength materials such as melamine. A few crystals of the acid are added to the water covering the paper sample and the solution brought to a boil. The solution is decanted, the sample washed several times with distilled water, and then given the usual dilute caustic procedure. Others have used a few drops of concentrated hydrochloric or sulfuric acid instead of sulfamic acid. The proprietary, Oakite EX-R*, has also been suggested for this purpose.

Pyroxylin-Treated Papers

The pyroxylin is removed by extraction with cellosolve (ethylene glycol monoethyl ether), ethyl acetate, or amyl acetate.

Dewaxing Procedure (for glassine)

Dewax the sample with the chemicals in the order listed: Petroleum ether, alcohol, ethyl acetate, and acetone.

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*Oakite Products, Inc., 19 Rector St. New York, N.Y., 10006

Disintegration of Parchment Papers

Method 1. The paper is added to a mixture of 25 ml. sulfuric acid (sp. gr. 1.84) and 25 ml. water cooled to 50-60°C.; when the paper begins to disintegrate, the mixture is stirred quickly and poured into a liter beaker two-thirds full of distilled water.

Method 2. The paper is treated with 50 ml. of a saturated solution of potassium permanganate (6.5 g. to 100 ml. water) for 45-60 minutes for lightly parchmentized and 60-75 minutes for heavily parchmentized paper; it is then washed, decolorized with 25 ml. of 5% oxalic acid containing a few drops of dilute sulfuric acid, washed with 43% sulfuric acid, and rinsed well before slides are prepared. Otherwise, the paper may be decolorized with sodium metabisulfite for five minutes, washed, and disintegrated (8).

Highly Colored Papers

If the paper is highly colored, remove the dye by one of the following methods and then disintegrate by the usual procedure. The treatment selected should be used with judgment and some knowledge of the characteristics of the dyes. By solution: Use alcohol, ammonia, acetic acid, or hydrochloric acid. By oxidation: Use nitric acid or bleach liquor. By reduction: Use hydrosulfite, stannous chloride, or hydrochloric acid with metallic zinc (3).

Preston (9) gives the following suggestions for stripping dyed fiber: The best stripping agent to use depends upon the fiber and the dyestuff in question. Direct dyestuff on cellulose fibers can frequently be stripped in 60% pyridine solution, or if this will not give sufficient stripping, then a solution of 10% pyridine and 5% sodium hydrosulfite should be tried, first without and then with the addition of a small quantity of sodium hydroxide. These stripping agents are more effective if used warm than cold. The same stripping agents can be used for silk and wool, but with these fibers the sodium hydroxide must be replaced with ammonia. A mixture of ammonia and hydrogen peroxide is also useful for removing the last trace of color from partly stripped animal fibers.

Cellulose fibers dyed with vat dyestuffs are best stripped with pyridine and sodium hydrosulfite, with the addition of a small amount of sodium hydroxide. The mixture generally works better if heated gently.

Dyed cellulose acetate fibers are easily stripped by 92% alcohol, with the addition of about 10% acetone. This solution must be used cold.

If the first treatment with the stripping agent does not remove enough color, the treatment should be repeated several times.

PREPARATION OF SLIDES

The methods of preparing slides vary considerably, from taking a sample out of the test tube with a needle (10), putting it on a slide, removing the water by pressing with filter paper, and then staining, to a much more precise and uniform method.

In the TAPPI method (T 401 sm-60) the fibers are transferred to the microscope slide by means of a dropper consisting of a glass tube about 10 cm. long with an internal diameter of 8 mm., with one end carefully smoothed, but not constricted, and the other end fitted with a rubber bulb. The tube should be graduated to deliver 0.5 ml. The details of the method are practically the same as the Institute method, which again is a modification of the Bright method.

In the Bright method (1) several clean three by one inch microscope slides are placed on a warm plate and 3 ml. of the fiber suspension are pipetted onto each slide. The suspension is distributed over the entire top of the slide with the aid of a dissecting needle, and, if care is used, will be held by surface tension. As the water evaporates, the fibers are distributed uniformly over the slide by tapping lightly with the needle. The tapping must be stopped before too much water has been evaporated, or the fibers will be picked up by the needle and bunched together, and poor distribution will result. This point is reached after about two-thirds of the water has evaporated when the fibers are just matting together and no longer float freely in the water. Then the slide should be tapped all over to distribute the fibers uniformly and the water should be allowed to evaporate to dryness without the slide being disturbed. The fibers will not change their relative position any further and will be uniformly distributed. The slide now has a definite quantity of fibers distributed over its entire surface.

Before staining, Bright found it convenient to moisten the fingers and remove from the slide all the fibers, with the exception of an area the size of the cover glass near each end. The fibers adhere to the slide very well, so that standard unstained slides can be prepared and kept indefinitely.

The Institute method of slide making (2) is a modification of the Bright method, which was intended to be used for the estimation method.

It is important that the microscope slides be thoroughly cleaned before use as it is impossible to get uniform fiber distribution if the slides are dirty, especially if greasy. Standard cleaning solution serves the purpose very well. After thorough washing, the microscope slides are stored in 50% alcohol. After the slide has been dried and polished, lines are drawn on it one inch from each end with a glass-marking pencil, a soft lead pencil, paint pencil, or aluminum stearate solution. The line helps to retain the fiber suspension inside a given area (one square inch) at each end of the slide. A repellent-type label tape* has been used to cover the center square-inch area of the slide. Dust, lint, and other dirt is removed from the slide with a small camel's hair brush, and the slide is then placed on a warm plate at 60°C. The warm plate must be absolutely level, must have a mat black top surface, and must have temperature control.

After the slides are laid on the warm plate, 0.5 ml. of the fiber suspension is placed on each end of the slide from a pipet made of glass tubing with an inside diameter of 6 mm. Care should be taken to keep the suspension well mixed while the samples are withdrawn. Some of the water is evaporated before the suspension is carefully tapped with a dissecting needle to distribute the fibers evenly inside the one-inch square areas on the ends of each slide. The slides are left on the warm plate until completely dried (Figure 119).

*Professional Tape Co., Riverside, Ill., 60546.

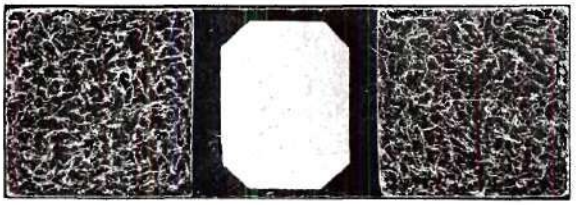


Figure 119. Standard Slide (Natural Size)

ALUMINUM STEARATE SOLUTION

Preparation

(a) To 600 ml. of distilled water add 15 g. of Ivory soap shavings and stir until the soap is completely dissolved. To the solution add 10 g. of c.p. aluminum sulfate, $Al_2(SO_4)_3 \cdot 18\ H_2O$. A white precipitate of aluminum stearate forms immediately. Stir with a glass rod until the precipitate coagulates into a waxlike mass. With the stirring rod lift out the precipitated aluminum stearate and place in a desiccator for 48 hours. Store in a well-stoppered bottle to be used as needed.

(b) To 50 ml. of benzene in a glass-stoppered bottle add 0.7 g. of desiccated aluminum stearate. Shake well daily until completely dissolved. This usually requires about 10 days. If desired the solution may be gently and carefully warmed to accelerate the preparation. If, after several weeks, it is found that the solution has lost some of its capacity as a water repellent, add a small piece of aluminum stearate to the solution and the condition will be corrected within a few hours.

Application

A practical method of applying aluminum stearate to the slide is as follows: Six slides or less are cleaned and arranged so that the long edges are in contact with each other. A six-inch wooden rule (which is one inch wide and has a metal edge) is placed on the slides, so that the rear edge of the rule is even with the ends. An ordinary pen holder fitted with an X-513 Hunt Silverine point is dipped in the aluminum stearate and the excess is allowed to flow off by touching the point to the edge of the bottle. A line is drawn on the slides along the metal edge of the rule, forming a thin line of aluminum stearate one inch from the ends. In like manner, a line of aluminum stearate is placed on the opposite ends of the slides. In order to obtain the best results it is advisable to wipe the pen point clean of any adhering aluminum stearate with a cloth after marking not more than two groups of slides.

This method of slide preparation may seem unnecessarily complicated to some workers, but a careful study of the details of the different methods used has shown that, thus far, this is the only method which gives any assurance of an even distribution of the different fibrous materials in a sample of paper.

PERMANENT SLIDES

It is often desired to make permanent slides of pulp fibers so as to have a record of fiber lengths or of the condition of fibers after refining. Such slides also furnish a convenient reference for the morphological details of the various fibers.

Unbleached Fibers

The basic dyes are satisfactory for staining unbleached fibers. Fuchsin, Malachite green, or methylene blue will give beautiful preparations, which will keep for years if protected from the light (11). After blotting the water from the fibers, two drops of a 1% solution of the dye is applied, allowed to stand for one minute, and the excess is blotted off. The stained fibers are then washed twice with distilled water, dehydrated gradually in alcohol, cleared in xylene, and mounted in Canada balsam. Other mounting media may be used after an appropriate clearing schedule.

Bleached Fibers

The basic dyes are not satisfactory for staining bleached fibers. Congo red stains bleached fibers, but the color fades. Safranin is permanent, but inconvenient to apply. Benzoazurin stains well and is permanent (11). The fibers are placed on the slide with two drops of a solution containing 1% benzoazurin and 1% sodium carbonate, and the slide is heated over a small flame; the sodium carbonate hydrolyzes, and the increased alkalinity turns the benzoazurin red. The slide is then removed from the flame and, upon cooling, the benzoazurin becomes blue. The excess dye solution is removed by blotting with filter paper, the dyed fibers are washed twice with distilled water, dehydrated, cleared, and mounted in Canada balsam or similar mounting medium.

An interesting variation of the staining with benzoazurin is the following: After the fibers have been stained and washed, as described above, the wash water is blotted off and, without waiting for the fibers to dry, they are distributed in a drop of sodium silicate solution and covered. The silicate soon hardens, giving a permanent slide that allows the structural details to be seen better than do the slides made with Canada balsam. At the same time, the alkali of the silicate changes the color of the benzoazurin to a pink similar to that of fuchsin (11).

There is one objection to slides made with Canada balsam. The refractive indices of the balsam and of the fibers are so nearly alike that many of the surface markings and other structural details of the fibers are not apparent. Diaphane is slightly better. Glycerin jelly is much better but is liable to spoil (11).

Recently, Strelis and Green (12) have recommended a hot aqueous solution of chlorazol black E as a stain for both unbleached and bleached fibers. Unlike many of the more common stains, such as safranin or benzopurpurin, which stain fibers or the larger structures adequately, but stain fibrils, fibril bundles, or pieces of thin primary wall material weakly or not at all, chlorazol black E does stain such structures deeply enough to make them easily visible in permanent micropreparations, under the microscope, or sharply rendered in photomicrographs. It

is almost impossible to overstain and it is not completely removed by normal washing techniques. Because it produces on fibers tones ranging from gray-black through various lighter gray tones, it makes possible photomicrography without the use of contrast filters. Also, permanent slides stained with this stain have great permanence.

STAINING

The methods of applying stains vary greatly, not only as regards the different types of stains used, but also in the procedure used for a particular kind of stain. The greatest differences occur in the application of the various potassium iodide-iodine-metallic salt stains and in the various dyeing methods for the differentiation of fibers.

In using the iodine stains, some analysts thoroughly mix the fibers and the stain on the slide with the aid of dissecting needles; others moisten the fibers with water or glycerin before applying the stain; and still others apply one part of the stain, blot off the surplus, and then apply the second part, and so on. In using other staining methods, e.g., the Alexander stain, the slide is stained with the dye, and the surplus stain is blotted off; next, the second and third parts of the stain are mixed on the slide; finally, the cover glass is put on and the surplus stain is drained off. All these staining methods, as well as the practice of using iodine stains in two or more steps, leave too much to the personal element as regards uniformly comparable results.

Many of the differences which are bound to occur when the slides are stained as described above, are overcome first, by using all iodide-iodine-metallic salt solutions as a single stain and, second, by drying the slides completely, applying three or four drops of the stain, covering the whole with a cover glass in such a manner as to avoid air bubbles, and then allowing the slide to stand at least one minute before draining off the surplus stain.

Care must also be taken that the unstained fibers on the slide are not touched by the worker's fingers. The analyst cannot help getting metallic salts of one kind or another on his fingers when working and, if he touches the fibers with the stained finger, they will absorb these salts and give rise to some very puzzling stain reactions.

When using dyes as reagents many analysts use the following procedure: The prepared slide is treated in the different baths or drops of the dyes are applied and allowed to remain on the slide for a certain time, the surplus dye is drained off, the slide is washed and the fixative or the other dye is applied. Since the application of dyes for staining is simply a dyeing process, in which the dyes are taken up according to the different chemical characteristics of the fibers and the dyes, it can be regarded as such and a certain amount of the fibers dyed in a beaker, according to the described methods. When the treatment is finished the fibers can be transferred to the slides and mounted in water or other media. In this manner, a much more uniform and reproducible dye differentiation of the fibers can be obtained.

For purposes of convenience the different staining methods can be divided into the following main groups:

1. Stains used primarily for determining the relative amount of groundwood or woody fibers in a paper. These are generally lignin reagents in one form or another.

2. Stains composed of different metallic salts and potassium iodide-iodine solutions which not only indicate the relative purities of the fibers but also the method of pulping and purification.

3. Dyes and salts used to determine the degree of cooking, bleaching, and purity of the fibers, and also for the purpose of determining unbleached sulfite and sulfate pulps.

4. Special stains used for the more positive identification of the various non-woody vegetable fibers, such as jute, manila, flax, hemp, etc.

5. Stains used for determining the sizing and coating of papers.

6. Stains used for fiber identification in the textile industry.

There is a large number of each type of these stains but in this chapter it is planned to consider only the iodine stains of more or less general utility; the miscellaneous stains of more restricted applicability are considered elsewhere, principally in the next chapter.

IODINE STAINS

The principal iodine stains have been known and used for many years with little or no variation in their composition. In general, the stains are either a potassium iodide-iodine, potassium iodide-iodine-sulfuric acid, potassium iodide-iodine-zinc chloride, or potassium iodide-iodine-calcium chloride solution. Other salts have been used, however, and more recently several combinations have been devised.

With a few exceptions, most of these stains differentiate only groundwood, chemical pulps, and rags, unless used by an expert analyst who is especially color sensitive. Furthermore, the color differentiations produced with these stains have been described so vaguely that each worker has had to devise his own system of identification.

It should be emphasized that all iodine stains (containing iodide-iodine) must be kept in dark or amber-colored bottles. The stopper should be designed to exclude air when not in use. The Institute of Paper Chemistry has used amber-colored dropping bottles of 1-oz. capacity for many years. In addition, these bottles are wrapped in black masking tape to further protect the stain from light. It should also be emphasized that these stains are corrosive in nature and will adversely affect microscope lenses.

POTASSIUM IODIDE-IODINE STAIN (13)

This stain is prepared from potassium iodide, 2.0 g.; distilled water, 20.0 ml.; iodine, 1.15 g.; and glycerin, 2.0 ml. The glycerin is added only to produce body and plays no part in the staining reaction.

The color reactions of this solution with various fibers are:

Brown: Cotton, linen, bleached hemp.
Yellow to yellow brown: Unbleached jute and straw, mechanical wood pulp.
Colorless to slightly gray: Bleached jute and straw, manila, esparto, chemical wood pulps.
Gray to brown: Adansonia.

POTASSIUM IODIDE-IODINE-SULFURIC ACID (14)

This stain is applied in two steps. The potassium iodide-iodine solution should be made so that a solution 3 cm. in depth has a ruby red color. The slide is stained with this solution and the excess is removed before the sulfuric acid is added. The sulfuric acid must be exactly 44.5° Bé. and is made by mixing 100 ml. of distilled water and 125 ml. of sulfuric acid (sp. gr. 1.85).

The color reactions of this stain with various fibers are:

Red violet to wine red: Cotton, linen, bleached jute.
Blue to blue gray: Wood and straw cellulose.
Gold to dark yellow: Groundwood and unbleached jute.

Although both Herzberg and Klemm gave von Höhnel credit for this stain, Pontes (15) in describing a modification of it, gave credit to Vétillart. Pontes stated that the above formula had an insufficient amount of iodine and an excess of acid. He proposed the following formula:

Solution A: Potassium iodide, 3 g.; distilled water, 100 ml.; and iodine to saturation.

Solution B: Sulfuric acid, 24 g.; distilled water, 16 ml.; and glycerin, 16 g.

The application of this modification of the stain and the color reactions produced are the same as the original.

HERZBERG STAIN

The fact that this stain was known to botanists long before it was used for fiber identification (16), is probably the reason why Maddox (17) did not give Herzberg credit for this stain, but stated that it is commonly called the "zinc chloride solution."

The stain used by Herzberg, and also by Klemm, was made as follows:

Solution A: Zinc chloride, 20 g.; and distilled water, 10 ml.

Solution B: Potassium iodide, 2.1 g.; iodine, 0.1 g.; and distilled water, 5.0 ml.

The color reactions produced on fibers by this stain are:

Light to dark claret: Linen, cotton, hemp.
Blue to claret: Esparto (both colors often occur on one fiber).
Blue to blue violet: Straw
Blue: Chemical wood pulps.
Olive green: Manila.
Yellow to colorless: Groundwood, unbleached jute, unbleached straw.

Another method for staining fibers (17) is to use the Herzberg potassium iodide-iodine solution as above and, after about a minute, remove the excess with filter paper. The stained material is then treated with one drop of sulfuric acid which has been diluted with four or five volumes of distilled water. This will produce a change in the colors resulting from the iodine solution as follows:

Violet red to wine red: Cotton, linen, bleached jute.
Blue to blue gray: Chemical wood pulps, straw.
Gold yellow to dark yellow: Groundwood, unbleached jute, raw chemical wood pulps.

The methods of making these stains and the variations of the composition to suit individual needs were so numerous that, before Merritt (18) published her article on the Herzberg stain, there was no standard for comparison.

This method has been adopted as a TAPPI standard (T 401 sm-60) and is as follows:

Solution A: Zinc chloride solution of 1.80 sp. gr. at 28°C., made by adding approximately 25 ml. of distilled water to 50 g. of dry zinc chloride (fused sticks in sealed bottles, or crystals).

Solution B: Dissolve 0.25 g. of iodine and 5.25 g. of potassium iodide in 12.5 ml. of distilled water.

Mix 25 ml. of Solution A with the entire Solution B. Pour into a narrow cylinder and let stand until clear (12 to 24 hours). Decant the supernatant liquid into an amber-colored, glass-stoppered bottle and add a leaf of iodine to the solution. Avoid undue exposure to light and air.

Although for special tests the Herzberg stain is sometimes modified by adding more zinc chloride to accentuate the blue, or more iodine to accentuate the red, modification is not recommended for normal use.

The stain must be renewed every month and, for very delicate differentiations, every two weeks. However, according to Minnear and Withrow (19), if properly prepared, the "life" of the stain is more than twelve months. Slides of rag, sulfite, and soda fibers stained with the Herzberg stain may be preserved for about two months by sealing the cover glass edges with paraffin to prevent changes in moisture and iodine content of the stained fibers. The rag fibers "bleach out" sooner than either sulfite or soda. Volatilization of free iodine from the Herzberg solution is probably the most common cause of deterioration of this reagent.

It is very important to use c.p. anhydrous zinc chloride sticks in sealed bottles, and never to accept any bottle which has been cracked or broken. Another important detail, which holds for any iodide-iodine solution, is that the necessary

amounts of potassium iodide and iodine must be thoroughly mixed by crushing and stirring before the water is added; sufficient water is added, drop by drop, to completely dissolve the potassium iodide and iodine.

Color reactions:

Red: Cotton, linen, bleached manila.
Blue: Chemical fibers low in lignin, such as wood, straw and esparto.
Yellow: Fibers high in lignin, such as groundwood, jute, and unbleached manila.

SUTERMEISTER STAIN

Sutermeister rightly remarked that both the iodine-sulfuric acid and the iodine-zinc chloride stains are corrosive, that they tend to dissolve the fibers, and that they must be used with caution. He recommended a stain, which has been designated the "Sutermeister stain," that is free from these defects (20). This stain consists of two solutions:

Solution A: Iodine, 1.3 g.; potassium iodide, 1.8 g.; and distilled water, 100 ml.

Solution B: A clear, saturated solution of calcium chloride.

In using this stain, Solution A is applied to the fibers moistened with water; after one minute, the excess stain is removed by blotting, and Solution B is added.

The colors produced by this stain are quite characteristic and although no two observers would classify them in the same manner, they may be described as follows:

Red brown: Rag stock, including cotton, linen, and hemp.
Dark blue black: Hardwood soda.
Violet: Bleached sulfite.
Yellow: Groundwood.
Greenish yellow: Jute, manila, and some of the less thoroughly cooked fibers in unbleached sulfite.

The staining characteristics of some of the fibers make it possible to determine the presence of unbleached sulfite in paper, but the reagent will not permit an estimation of its amount. Unfortunately, this stain cannot be used to determine sulfate fibers.

According to Korn (21), the Sutermeister stain colors not only hardwood bleached soda dark blue, but also straw and esparto. It colors groundwood more intensely and redder, and the chemical pulps a weaker blue and, occasionally, gray blue. Thus, in mixture the groundwood tends to be overcounted. In individual cases the stain may be used to advantage, but in Korn's opinion it cannot be substituted for iodine-zinc chloride solution in fiber microscopy. It is interesting to note, however, that for more than twenty years Graff used the Sutermeister stain and found it to be far superior to the Herzberg stain.

The Sutermeister stain has many advantages over other iodine stains but, because the iodine absorption of cellulose fibers is related to their respective moisture contents (22), the necessity of moistening the fibers with water before applying Solution A causes an uneven absorption of the iodine. Furthermore, since this is a staining method involving two steps, Graff modified the stain to a one-solution stain, the "A" stain, or modified Sutermeister stain (23). Until 1932, Graff considered this stain to be far superior to any of the previously existing stains.

GRAFF "A" STAIN (MODIFIED SUTERMEISTER STAIN)

Solution A: Calcium chloride of 1.36 sp. gr. at 28°C. is made by adding about 100 g. of calcium chloride to 150 ml. of distilled water.

Solution B: Potassium iodide, 0.9 g.; iodine, 0.65 g.; and distilled water, 50 ml.

To 45 ml. of Solution A are added 5 ml. of Solution B. The solutions are mixed well and poured into an amber-colored, glass-stoppered bottle; a leaf of iodine is added and the solution is kept in the dark when not in use.

JENKE STAIN (24)

Solution A: 50 ml. of saturated magnesium chloride to which are added 2.5 ml. of Solution B.

Solution B: Potassium iodide, 2.0 g.; iodine, 1.15 g.; and distilled water, 20 ml.

Color reactions:

Brown: Rag.
Blue violet: Straw.
Colorless: Chemical wood pulps.
Yellow: Groundwood and raw jute.

This stain has no practical value, however, not even in its modified form, Graff "D" stain (23).

ALEXANDER STAIN (25)

Solution A: Dissolve 0.2 g. of Congo red in 300 ml. of distilled water.

Solution B: Dissolve 100 g. of calcium nitrate in 50 ml. of distilled water.

Solution C: Standard Herzberg stain.

The fibers on the slide are covered with two drops of Congo red solution and allowed to stand for one minute; the excess dye is removed and the slide dried;

the slide is then covered with three drops of the calcium nitrate solution and allowed to stand for one minute; one drop of the Herzberg stain is added to the nitrate solution on the slide, thoroughly mixed with it, and a cover glass mounted. The colors seem to be stronger if the stain is allowed to stand for three or four minutes before covering.

This stain was discussed at a Sectional Meeting on Paper Testing in 1924 (26), at which it was recommended as the most satisfactory stain for determining the proportions of sulfite and soda pulps in paper.

According to Korn (27), the color reaction is characteristic for unbleached and bleached sulfite pulps from coniferous woods, soda and sulfate fibers from hardwoods, esparto, and groundwood. On the other hand, the soda and sulfate fibers from coniferous woods give very different reactions.

In many softwood pulps Korn found that only a part of the individual fibers had the proper color, and in many cases the intermediate colors were confusing and made differentiations difficult. Experiments were performed with pulps which had been cooked, bleached, and beaten to different degrees. Bleaching and beating differences had little effect, but the different degrees of cooking gave variations in the color reactions. These results showed that the staining method was quite unreliable when used on any type of coniferous wood fibers.

Korn suggested a simplification of the stain by eliminating the Congo red and doubling the strength of the calcium nitrate solution. The fibers on the slide are first covered with three drops of the calcium nitrate solution and allowed to stand for one minute, after which one drop of the Herzberg stain is added and mixed with the nitrate; after standing for a few minutes, the cover glass is added. Softwood sulfite, sulfate, and soda pulps are colored brownish red with a slight violet haze, rag gives a similar color but is more tinted, and groundwood is colored yellow. The color reactions of straw and esparto differ greatly from the reaction of sulfite fibers. This method is helpful when a mixture consists of manila and cotton, because the brillant red color of the cotton fibers is dominant.

SELLEGER STAIN (28)

Solution A: 100 g. of calcium nitrate in 90 ml. of distilled water, to which are added 3 ml. of Solution B.

Solution B: Potassium iodide, 5.0 g.; iodine, 1.0 g.; and distilled water, 50 ml.

Color reactions:

Brown: Cotton.
Dull violet: Bleached softwood.
Blue: Straw and hardwood.
Dull yellowish: Unbleached softwood.
Yellow: Groundwood.

Geohegan (11) states that stains recommended for differentiating between sulfite and soda fibers distinguish between softwoods and hardwoods and not between cooking methods and that Selleger stain will do this more easily than

Alexander stain. According to an earlier article (29), Selleger stain colors the fibers as follows: Bleached softwood, light rose; unbleached softwood, clear yellow; poplar and birch soda, violet blue; esparto, blue; manila, wine red; linen and cotton, rose or brownish; groundwood, jute, and straw, deep yellow. The differences between many of the above colors are so slight that they are hard to distinguish. The blue of hardwood fibers and that of esparto, however, are easily distinguished from the pale colors of the other fibers.

The following two methods can be used for preparing Selleger stain so that it will retain its strength.

Method 1. 100 g. of calcium nitrate are dissolved in 50 ml. of distilled water and 3 ml. of a solution containing 8.0 g. of potassium iodide in 90 ml. of water are added, followed by one gram of iodine; after one week the stain is ready for use.

Method 2. A solution of 0.267 g. of potassium iodide in 53 ml. of distilled water is treated with one gram of iodine and allowed to stand for two weeks; the mixture is shaken each day, so as to saturate the solution with iodine. One hundred grams of calcium nitrate are dissolved in this solution, when it is ready for use.

By saturating a solution containing one gram of potassium iodide in 198 ml. of distilled water with iodine, a stock solution can be made, to which it is only necessary to add calcium nitrate in the proportion of 100 g. to 53 ml. of stock solution.

In the opinion of many workers, however, the Sutermeister stain, even in its original form, is far superior to any of the modifications of the Selleger stain.

FERRIC CHLORIDE-FERRICYANIDE-CALCIUM NITRATE STAIN (30)

Solution A: 2.7 g. of ferric chloride in 100 ml. of distilled water.

Solution B: 3.29 g. of potassium ferricyanide in 100 ml. of distilled water.

Solution C: 100 g. of calcium nitrate in 50 ml. of distilled water and 3 ml. of a solution made by dissolving 8.0 g. of potassium iodide in 90 ml. of distilled water and adding one gram of iodine, and allowed to stand for one week before using.

Ten milliliters of Solution A are mixed with 10 ml. of Solution B and the slide is placed in the stain for three minutes at 20°C. The slide is washed by dipping in distilled water three times and dried by evaporation. Three drops of Solution C are placed on the fibers, allowed to stand for one minute, and then covered with a cover glass. Bleached sulfite stains pink; unbleached sulfite, blue; bleached hardwood soda, violet; groundwood, greenish yellow; and rag, wine red.

OTHER CHLORIDE STAINS

Other constructive efforts to find an iodine stain which would differentiate among the various pulps have been made from time to time. Strasburger and Koernicke

(31) in 1923 suggested aluminum and stannic chlorides, among other metallic salts, and Wisbar (32) in 1920 stated that microscopic examination of paper was facilitated by the use of solutions of stannic chloride and iodine, calcium chloride and iodine and, particularly, aluminum chloride and iodine.

The aluminum and stannic chloride solutions are made by dissolving 0.1 g. of iodine and 0.5 g. of potassium iodide in a minumum quantity of distilled water, and increasing the volume to 10 ml. by the addition of a saturated aluminum chloride or stannic chloride solution of 1.53 sp. gr. The fibers on the slide are moistened with dilute glycerin, a few drops of aluminum chloride-iodine solution are added, and the excess removed with blotting paper; this is followed by a drop of zinc chloride-iodine solution, and finally mounted under a cover glass.

In 1925, Lee (33) suggested a number of combinations of magnesium, calcium, and zinc chlorides in different proportions with potassium iodide-iodine, and stated that they were superior to the foregoing stains in so far as bleached fibers were concerned and could be applied with great ease.

All of these iodine stains, even at their best, can differentiate only among rag, groundwood, sulfite (with faint differentiation between unbleached and bleached) and, in certain instances when used by trained observers, also sulfate pulps. In the last three or four decades, however, a large number of new and highly purified pulps with very special properties have been introduced as papermaking materials. The special qualities of these pulps made it imperative that a staining method be developed which would permit the differentiation of such pulps, as well as the ordinary chemical pulps.

GRAFF'S OBSERVATIONS

About 1930 Graff observed that, if softwood and hardwood sulfites and kraft were treated with a certain percentage of sodium hydroxide and sodium hypochlorite, the fibers, when treated with the "A" stain, would give a complete rag reaction. When treated with the same percentage of sodium hydroxide alone, or a little less caustic and somewhat less sodium hypochlorite and stained with the "A" stain, the fibers gave a stain reaction a little closer to rag than to sulfite. This fact, together with the statements by Sutermeister (20) that less thoroughly cooked sulfite fibers gave a greenish-yellow reaction with his stain, and by Korn (27) that different degrees of cooking of chemical pulps gave different color reactions with Alexander stain, Graff's own observations of similar evidence, and a careful study of color reactions produced on various fibers by the other iodine stains, led to a belief that a single stain embracing the good points of all the other stains could be developed. Graff believed that this stain, which should differentiate among types of chemical pulp and degrees of cooking, bleaching, and further purification, could be made by combining certain amounts of metallic salts and a potassium iodide-iodine solution.

The results of this research by Graff were his "C" and "F" stains (23), combinations of different proportions of the same metallic salts and iodide-iodine solutions. At first it was difficult to decide which was superior, but, after several years of use, Graff, and others with proper instruction and experience in the use of these stains, preferred the "C" stain.

GRAFF "C" STAIN (23)

The "C" stain is prepared from the following solutions: 20 ml. of aluminum chloride solution (1.15 sp. gr. at 28°C.), 10 ml. of calcium chloride solution (1.36 sp. gr. at 28°C.), 10 ml. of zinc chloride solution (1.80 sp. gr. at 28°C.), and 12.5 ml. of iodide-iodine solution, made by adding 50 ml. of distilled water to 0.9 g. of potassium iodide and 0.65 g. of iodine. The potassium iodide and iodine are first thoroughly mixed and crushed together and then the required amount of water is added drop by drop with constant stirring.*

These solutions are thoroughly mixed, poured into a tall, narrow vessel, and placed in the dark. After 12 to 24 hours, when the precipitate has settled, the clear portion is pipetted into a dark, glass-stoppered dropping bottle and a leaf of iodine is added. The stain is best kept in the dark when not in use.

GRAFF "F" STAIN (23)

The "F" stain is made by mixing 20 ml. of aluminum chloride solution, 6.5 ml. of calcium chloride solution, 6.5 ml. of zinc chloride solution, and 14.5 ml. of potassium iodide-iodine solution. Each of these solutions is prepared as given under the directions for "C" stain.

The fiber separation obtained with the Herzberg, the "A", and the "C" stains are compared in Table XXIV, and the color reactions of these stains are shown in Graff's Color Atlas (34).

WILSON STAIN (35)

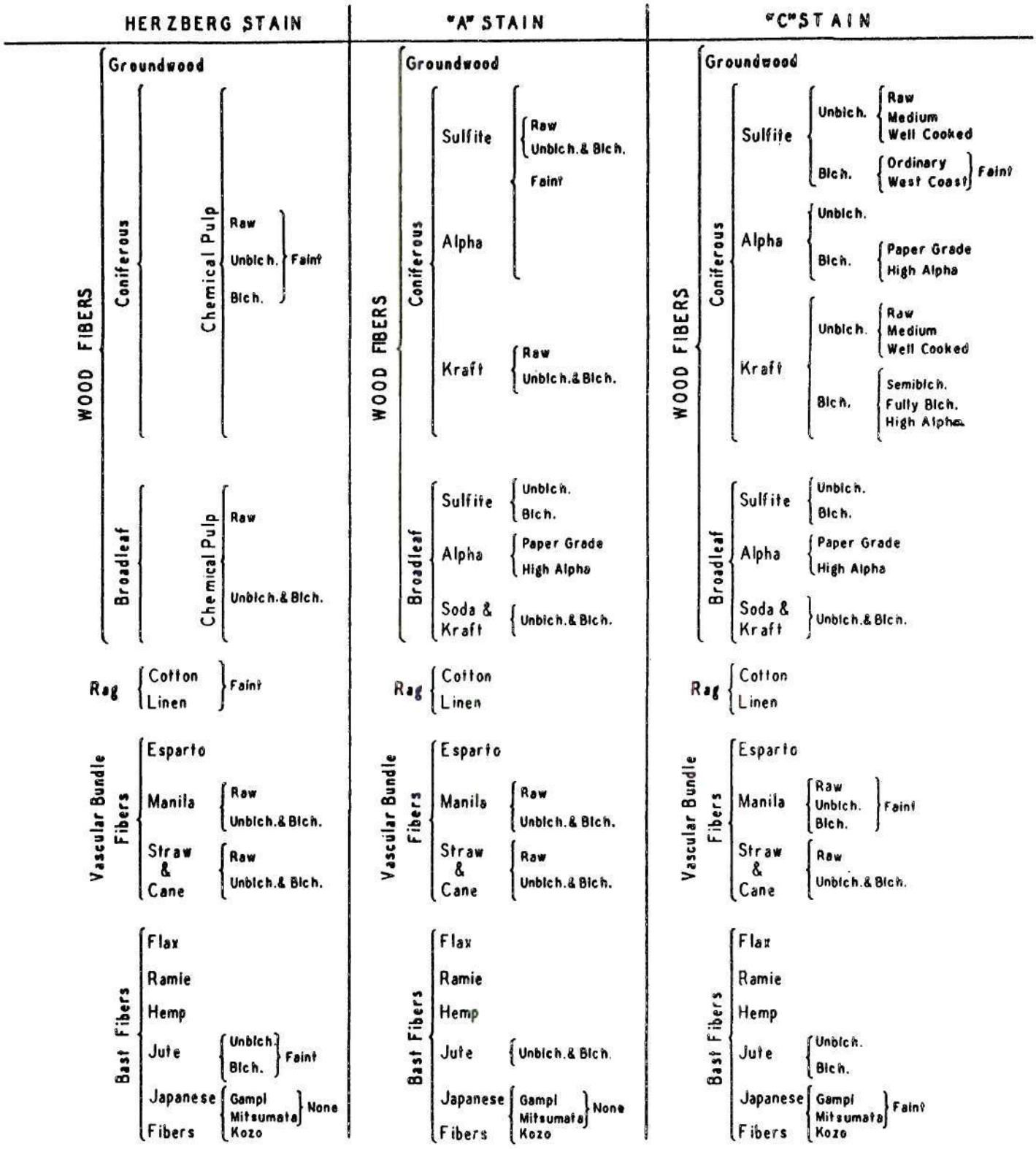
The Wilson stain is prepared as follows: 0.8 g. of iodine crystals, 35 g. of cadmium iodide, and 50 ml. of distilled water are heated to 110°F. (43°C.), the iodine crystals being crushed with a stirring rod. The solution is then treated with the following, in the order mentioned, with stirring: 90 ml. of distilled water, 7 ml. of 37% formaldehyde, 70 g. of calcium nitrate (with four molecules of water), and 20 g. of cadmium chloride (with 2.5 molecules of water). In all cases, chemically pure chemicals are used. The stain may be used immediately after preparation.

The same general color reactions are obtained as with the "C" stain although some differences are observed on certain pulps (36). The Wilson stain, because of the tendency of the color reactions of sulfite pulp to be more tinted and toward the yellow side than with the "C" stain, gives less distinct differentiation among raw, medium, and well-cooked fibers. Although the Wilson stain differentiates between sulfite and kraft pulps, this distinction is less sharp and more toward the sulfite reaction than with the "C" stain. Fibers treated with Wilson stain appear to retain the original color longer than when treated with "C" stain (36).

*The "C" stain, as well as many of the other stains mentioned in this book, may be purchased from The Institute of Paper Chemistry, Appleton, Wisconsin.

TABLE XXIV

FIBER DIFFERENTIATION WITH IODINE STAINS



QUALITATIVE AND QUANTITATIVE DETERMINATIONS

QUALITATIVE DETERMINATION

Since the fibers on the microscope slide prepared by the standard method occupy a one-inch square on each end of the slide (Figure 119), it is advisable to use cover glasses of nearly the same width (25 mm.). The Institute analysts use 25 mm. square, No. 2 cover glasses for routine fiber analyses.

When the slide has been stained, the cover glass placed on, allowed to stand for one minute, and the surplus stain drained off by tilting the long edge of the slide against a blotter, the slide is placed on the microscope stage and with the light from a daylight fluorescent lamp properly centered and focused, it is first examined for the different fiber constituents present in the mixture. It is important that the edges of the condenser iris diaphragm are just visible in the field of view so that the amount of light coming through the microscope will be fairly consistent with the same source of light.

The fiber constituents are reported as follows:

Groundwood;
Softwood unbleached sulfite, unbleached alpha, and unbleached kraft;
Softwood bleached sulfite, bleached alpha, semibleached and fully bleached kraft;
Hardwood unbleached sulfite, unbleached soda, unbleached kraft, unbleached neutral sulfite, and unbleached cold soda;
Hardwood bleached sulfite, bleached alpha, bleached soda, bleached kraft, bleached neutral sulfite, and bleached cold soda;
Rag (cotton and/or linen);
Esparto;
Unbleached or bleached jute;
Unbleached or bleached manila;
Raw, unbleached, and bleached straw;
Japanese fibers such as mitsumata, gampi, or kozo;
And other fibers which may be present.

Comments should always be made also as to softwood or hardwood groundwood, and the degree of cooking and the extent of the purification of the fibers as well as to type and degree of refining of the furnish as a whole.

The efficacy of the several stains in permitting the analyst to identify pulp fibers in the above list varies greatly and no one stain will enable one to make all separations. There are instances, too, where it is very difficult, if not impossible, to make separations between fibers with the general stains, or any of the known specific stains. As examples, hardwood unbleached and bleached kraft, and bleached kraft and bleached neutral sulfite semichemical may be mentioned.

QUANTITATIVE DETERMINATION

When the various components of the fiber furnish have been identified, the analyst usually wants to know the relative amounts of those kinds present. With

the aid of the mechanical stage the fiber field is adjusted under the microscope to about 2.5 mm. from the upper or lower edge of the cover glass and to the extreme left or right edge of it.

The stage is then gradually moved in such a way that the slide goes from left to right or vice-versa. There are two procedures which may be followed depending on the wishes of the analyst. The older method has been to count the fibers of one kind as they pass the center of the cross hairs or the end of the pointer in the eyepiece or perhaps to count two fiber types on each pass depending on the complexity of the furnish. It has been the practice of the author, however, to identify and count each fiber as it meets the pointer, because in this way no fibers are overlooked when the separations happen to be close. After the fibers have been counted in one line across, the slide is moved 5 mm. up or down, and the counting of the fibers continued until all the fibers have been counted in five lines 5 mm. apart on the slide (Figure 120).

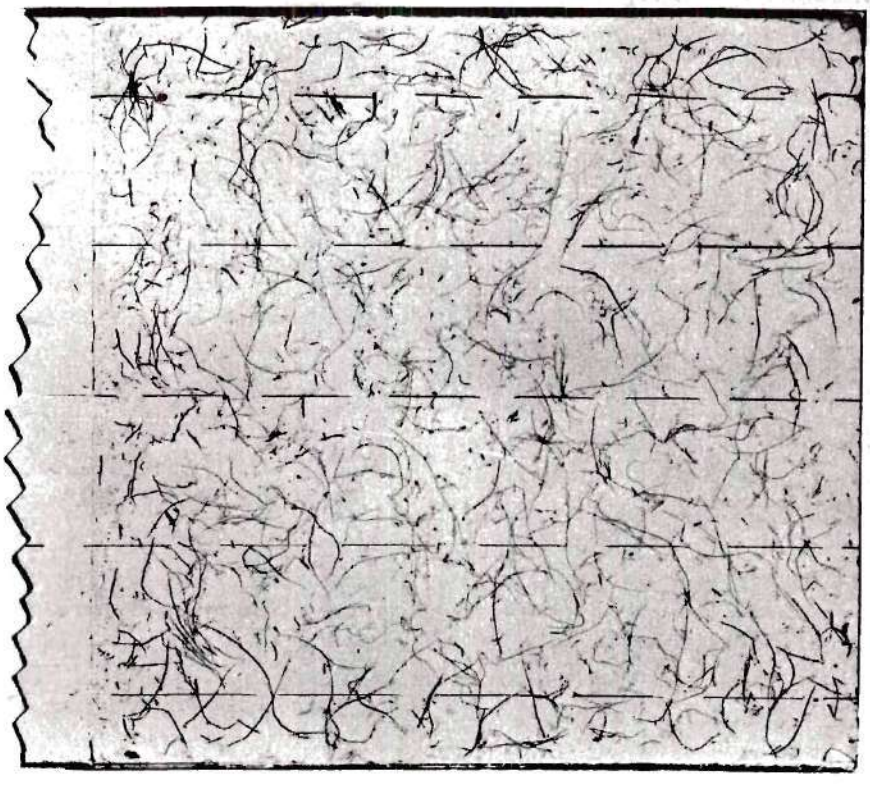


Figure 120. Quantitative Determination
(Dashed Lines Show Five Lines of Counts)

If a fiber crosses the center of the cross hairs more than once, it is counted each time; but if part of the fiber remains parallel with the center for some time, it is counted only once.

In fiber bundles or chunks of groundwood, every fiber readily visible in the bundle is counted as it passes under the center. Very fine fragments are ignored, but larger fragments are kept in mind, so that when two or three of the same kind occur in the same line or field, they may be mentally grouped as one unit. Some experienced analysts have suggested that rather than count fractions and mentally group them to give a whole number, that any fiber of reasonable length be counted as one, ignoring fines, and using 0.1 mm. as an approximate limit. It is very desirable for the analyst to analyze furnishes of known groundwood composition occasionally in order to retain his proficiency in counting groundwood.

If one uses the older method of counting it is well, if a furnish contains two types of fibers which are difficult to differentiate, to count first the one which can be distinguished more easily and then on a second traverse to count every fiber of both kinds until a total of the first count is reached; the remaining count of the two kinds will be the total number of the second kind.

It is time saving to use a tally counter if a large number of analyses must be made. With the older method the counter may be held in one hand to count one kind of fiber at the same time a second is counted mentally; in this way, two kinds of fibers can be counted each time the microscope field is brought across the stage. A better way to do this, however, is to have a bank of tally counters, each counter used for one fiber type. Units having four or five counters may be purchased, or, if desired, a person may combine any number of individual counters on a rack of some type.

The total count for each kind of fiber is then multiplied by its respective weight factor and the percentage calculated. In reports for court cases and in disputes, both the percentage by number and the percentage by weight should be given.

WEIGHT FACTORS

When equal weights of two pulps (e.g., one gram each of rag and soda pulp) are mixed thoroughly and standard slides are prepared and examined under the microscope, it is observed that there are nearly twice as many soda fibers present as rag fibers. Therefore, if rag is given a value of 1.0, soda would have a value of 0.5. These relative values have been designated weight factors. This fact was first demonstrated by Spence and Krauss (37) in 1917; they found that the weight factors for different pulps varied considerably. Further investigations of the relative weight factors were made by Minor and Graff (38) in 1925, by Landes (39) in 1932, and again by Graff (40) in 1933. A comparison of the results of these investigations revealed the following facts: (1) The hardwood soda weight factors obtained by all investigators check fairly well as a group; the different woods also give fairly consistent values; (2) The hardwood sulfite factors seem to be higher than the soda factors, whereas the hardwood kraft pulp factors are still higher; (3) Both Landes and Graff found that the weight factors for Scandinavian softwood pulps were higher than those for eastern softwood pulps; the values for west coast pulps were found to be somewhat higher than those for Scandinavian pulps. A study of these data suggested the proposed weight factors used at The Institute of Paper Chemistry for several years.

These factors gave fairly satisfactory results for general analyses of paper furnishes, but the question was often raised as to the effect of beating on the

weight factors of the different types of fibers in paper. In order to answer this question, Graff studied several selected pulps, each of which was beaten to three different degrees of freeness (41).

Fifty-fifty mixtures were made of the rag pulp and each of the other pulps, using all possible combinations of relative freeness. The mixtures were thoroughly disintegrated and made into sheets. A sector of about one-eighth of the total area of the sheets was cut out and disintegrated in water; standard slides were prepared from these pulp mixtures and the counts of the components made in the usual manner. The results showed that there is no appreciable difference in these factors for a given pulp or for a combination of pulps (when beaten to different degrees of freeness) and that the standard deviation of the average weight factor in each group of pulps is smaller than that for two different pulps in the same group under the same conditions of beating.

The results confirmed previous findings (38-40) that the weight factors of Scandinavian softwood pulps are higher than those of eastern softwood pulps and that the weight factor of west coast softwood (hemlock) pulps is still higher. But the most outstanding result was that the weight factor of southern unbleached kraft is 1.556, as compared with the average weight factor of 0.898 for midwestern and Norwegian unbleached kraft. The values obtained showed that the earlier proposed weight factors required very little modification, except that those determined for western sulfite (hemlock) and for southern kraft pulps must be used when they are present in a paper furnish.

Later Graff investigated the weight factors for a number of Asplund Defibrator and Chemi pulps, different gum soda pulps, jute, ramie, kozo, and mitsumata pulps as well as manila, pineapple and straw pulps, esparto, bamboo, and cotton linters (42). These investigations did show that the standard deviations of the weight factors for the Asplund Defibrator and Chemi pulps are fairly large and that those for the gumwood chemical pulps, although much smaller, are relatively large as compared with the results shown for some of the other pulps and the results of previous investigations. This would be expected, however, considering the relative coarseness of the fibers.

The weight factor for kozo, although relatively uniform for the different degrees of freeness, not only gives a large standard deviation, but this deviation increases with the decrease in the freeness. The reason for this is probably the uneven separation of the outer sheath covering from the main fibers; it may also depend upon the grade of kozo investigated.

An examination of the results for manila fibers shows that the weight factor depends upon the grade of manila and that, the better the grade, the higher is the factor. However, it is not easy to differentiate manila and sisal in a fiber mixture; therefore, the average obtained from manila and sisal will serve the purpose with a reasonably small standard deviation.

A very interesting fact is observed in the weight factor for cotton linters. Although the standard deviation for the different degrees of freeness is relatively small, the weight factors decrease with a decrease in the freeness so that, at a freeness of 405, the linters have a weight factor equal to that accepted for rag stock. Thus, the weight factor for virgin cotton fibers is considerably larger than that accepted for rag fibers; this factor is decreased, not only by manufacturing into fabrics and through use and wear, but also by beating of the original fibers (42, 43).

Many of the weight factors in general use were determined by Graff for the species commonly used at that time for making these grades of pulp. To a great extent they depend on the size of the cell elements included in the count. The species has a considerable effect on the weight factor, especially among the hardwoods, and the Institute (44) has a continuing program of determination of weight factors on authentic species.

A list of suggested weight factors is included in Table XXV. These factors should be used as a guide and, whenever possible, factors should be determined for the actual pulps used in the sample being analyzed. An experienced analyst can use the width of the fibers as a guide in determining the correct weight factor to use (45). Weight factors are directly related to the coarseness or decigrex of the pulp as determined by TAPPI method T 234 su-64.

Ranger (50) has proposed a method for determining weight factors, based on incorporating a small known percentage of dyed fibers in a handsheet, counting under the microscope the number of these dyed fibers encountered in a line-traverse of known distance on a portion of the handsheet, and calculating the relative grex or international denier value. This grex value is proportionate to the weight factor determined in the usual manner.

REPORT

The proportion of the various fibers found shall be reported to the exact whole percent, and one percent or less shall be reported as traces. The reason that this is more logical than to report the different fiber constituents of the paper furnish to the nearest five percent, as many do, will be seen from the following two random samples:

Example 1

Suppose a sample of paper is composed of 45% of A and 55% of B.

	Actual Count, %		Report, %	
	A	B	A	B
Analyst 1	42	58	40	60
Analyst 2	48	52	50	50

The analysts are actually within plus or minus three percent of the fiber composition of the paper, but when the report is made to the nearest five percent the difference between the two is ten percent, which in case of a dispute is quite some difference.

Example 2

Fiber Furnish	Actual, %	Counted, %	Reported, %
Rag	30	33	35
SW kraft	40	43	45
SW sulfite	20	18	20
HW sulfite	10	6	5
Total	100	100	105

TABLE XXV

WEIGHT FACTORS FOR PULPS

RAG	1.00
GROUNDWOOD	1.30
SOFTWOODS	
Asplund Defibrator and Chemi Pulps	1.91
Unbleached and bleached sulfite and kraft (except Douglas-fir, southern pine, and western hemlock)	0.90
Douglas-fir	1.50
Southern pine	1.55
Western hemlock	1.20
Alpha	0.70
HARDWOODS	
Asplund Defibrator and Chemi Pulps	1.26
Gumwood chemical pulps	1.00
Unbleached and bleached soda, sulfite, kraft, and neutral sulfite semichemical	0.60
Alpha	0.55
BAST FIBERS	
Linen rag	1.08
Flax bast fiber	0.56
Flax shives	0.33
Hemp	0.41
Jute, unbleached and bleached	0.55
Ramie	0.51
Kozo	1.38
Mitsumata	0.40
LEAF FIBERS	
Manila and sisal	0.60
Pineapple	0.60
GRASS FIBERS	
Straw, Asplund Chemi Pulp	0.53
Straw, raw	0.60
Straw, unbleached and bleached	0.36
Unbleached bagasse for boards	0.90
Unbleached and bleached bagasse for papers	0.80
Cornstalk	0.54
Cane, bleached	0.75
Bamboo, unbleached and bleached	0.56
Esparto	0.53
SEED HAIRS	
Cotton linters	1.25
ANIMAL FIBERS	
Wool hairs (46)	3.10

These results show that the percentages counted are considerably more accurate than the reported results, and they also show that the total of the reported results is five percent too great. The possibility of eliminating such a discrepancy by use of a plus and minus system suggests itself. For example, 18 could be reported as 20-, 19, 20, and 21 as 20, and 22 as 20+.

PRECISION

In TAPPI method T 401 sm-60 the following comments are made on precision. The precision depends on the skill and experience of the operator and on the selection of proper weight factors. Competent workers are expected to be able to check the composition of a furnish that is not too complex within the following tolerances:

Percent of Given Fiber in Total Furnish	Tolerance
Less than 20	±2
20 to 30	±3
30 to 40	±4
40 to 60	±5
60 to 70	±4
70 to 80	±3
Over 80	±2

It is emphasized that to achieve this precision, authentic pulp mixtures should be examined from time to time, to insure that sound judgment is exercised when including or rejecting debris in the count. Under ideal conditions, with weight factors determined on the pulps being used, it is possible for experienced analysts to check the composition of a furnish within half the stated limits [e.g., see the analyses of several types of paper in Graff (2)].

The precision is, of course, a function of the number of fibers counted and confidence levels of 95% probability can be calculated from a standard formula. An optimum precision that can be expected is determined. In practice, the discretion and selectivity required of the analyst usually causes the precision and accuracy to be poorer particularly with highly refined furnishes (47, 51).

GRADING OF FIBER ANALYSTS

As experience has shown that only a relatively small percentage of people are naturally equipped to become good fiber analysts, it is necessary in order to judge the accuracy of qualitative and quantitative analysis of the fiber furnish of paper, that the management of a mill, particularly in case of dispute, should have a standard for evaluating the general ability of the analyst. It is of importance to know to what degree the analyst is able to identify and differentiate the types and variations of the present-day papermaking fibers and the relative accuracy with which he determines quantitatively the presence of these fibers in a paper furnish. Are his quantitative determinations usually made with an error of less than five percent, or are his analyses more or less erratic? Is he able to differentiate accurately among the different types of pulp fibers and determine the relative degree of cooking and purification of them?

To be able to compare the relative abilities of different analysts in these respects, two methods of grading are necessary, one for the purpose of determining the degree of accuracy of the qualitative analysis, and the other for determining the reliability of the quantitative determinations.

QUALITATIVE ANALYSIS GRADING

To find a means to evaluate the results of qualitative analysis of a fiber furnish would appear at first to be somewhat of a problem, but when the different fiber constituents are evaluated according to the relative ease or difficulty with which they can be differentiated, using the proper stains or reagents, it is possible to construct a fair standard for such an evaluation. A suggested layout of such a scale is illustrated on page 242, but this may have to be altered considerably for individual needs or demands. A valuation chart which is more demanding has been used for many years in grading both regular and special students at the Institute.

QUANTITATIVE ANALYSIS GRADING

Libby (48) found that, on the average, one-third of his students made their analysis with an average error of less than five percent, one-third with errors between five and ten percent, and one-third with errors of ten percent or more. Graff (49), for the purpose of finding a fair method for the evaluation of quantitative analysis, first made a tentative evaluation of the different percentages of errors (Figure 121).

Based on this grading the results of quantitative analysis for 97 regular and 46 special students, each making analyses of 45 different pulp mixtures, gave the results shown in Figure 122, which indicate that the average error of four percent or less should be regarded as excellent, errors from four to eight percent as passing, from eight to ten percent as unsatisfactory, and above ten percent as failures. However, since 70% is generally accepted as a passing grade, the percentage evaluation of the errors, as first proposed, had to be modified as shown in Figure 121.

In evaluating the total results of the analyst, the gradings of his qualitative and quantitative determinations are totalled and divided by two.

Every analyst will make some errors, both qualitatively and quantitatively. These errors may be uniformly large or small, or more or less erratic; therefore, the evaluation of an analyst cannot be determined by a single set of analyses, but by the uniformity of his results over a stated period of time. If the general results of two different analysts, graded by this method, should run as shown in the following examples, one would have a rating of 94%, and the other a rating of 79%.

In the settlement of a dispute there would, of course, be quite a difference in the weight of arguments based on the results of Analyst No. 1 and Analyst No. 2. This demonstrates the need for a fair method of grading fiber analysts comparatively before their reports can be used as convincing evidence in disputes.

PERCENTAGE GRADING OF QUALITATIVE ANALYSIS OF PAPER FURNISHES

			SOFTWOOD										HARDWOOD						MISCELLANEOUS FIBERS															
			GROUNDWOOD	SULFITE		ALPHA			KRAFT				SODA	SULFITE		ALPHA	KRAFT		RAG		FLAX	MANILA		JUTE		HEMP		ESPARTO	BAGASSE	STRAW		JAPANESE FIBERS
				Unbleached	Bleached	Unbleached	Bleached	Special Treated	Unbleached	Semi-bleached	Fully Bleached	Special Treated		Unbleached	Bleached		Unbleached	Bleached	Cotton	Linen		Unbleached	Bleached	Unbleached	Bleached	Hemp Fibers	Hemp Hurds			Unbleached	Bleached	
	GROUNDWOOD		100																														
SOFTWOOD	SULFITE	Unbleached		100	80	90	50	20	80	70	60	20																					
		Bleached		80	100	60	90	20	50	60	60	40																					
	ALPHA	Unbleached		90	60	100	50	20	80	50	40	20																					
		Bleached		52	90	50	100	80	40	40	40	20																					
		Special Treated		20	20	20	80	100	20	20	40	80																					
	KRAFT	Unbleached		80	50	80	40	20	100	80	60	20																					
		Semi-bleached		70	60	50	40	20	80	100	80	20																					
		Fully Bleached		60	60	40	40	40	60	80	100	60																					
		Special Treated		20	40	20	20	80	20	20	60	100																					
HARDWOOD	SODA												100	80	60	40	90	90			60							60					
	SULFITE	Unbleached											80	100	80	60	80	80			40							40					
		Bleached											60	80	100	80	70	70			20							20					
	ALPHA												40	60	80	100	50	50															
	KRAFT	Unbleached											90	80	70	50	100	90			40							40					
		Bleached											90	80	70	50	90	100			40							40					
MISCELLANEOUS FIBERS	RAG	Cotton																	100	90		70	70			80	60						
		Linen																	90	100	70	70	70			90	70						
	FLAX												60	40	20		40	40		70	100	40	60			70	80	80	70	70	80		
	MANILA	Unbleached																	70	70	40	100	90	80	80	80	70						
		Bleached																	70	70	60	90	100	80	80	80	70						
	JUTE	Unbleached																				80	80	100	90	70	60						
		Bleached																				80	80	90	100	70	60						
	HEMP	Hemp Fibers																	80	90	70	80	80	70	70	100	90						
		Hemp Hurds																	60	70	80	70	70	60	60	90	100	70					
	ESPARTO												60	40	20		40	40			80						70	100	60	40	40		
	BAGASSE																				70							60	100	80	80		
	STRAW	Unbleached																			70							40	80	100	90		
		Bleached																			80							40	80	90	100		
	JAPANESE FIBERS																																100

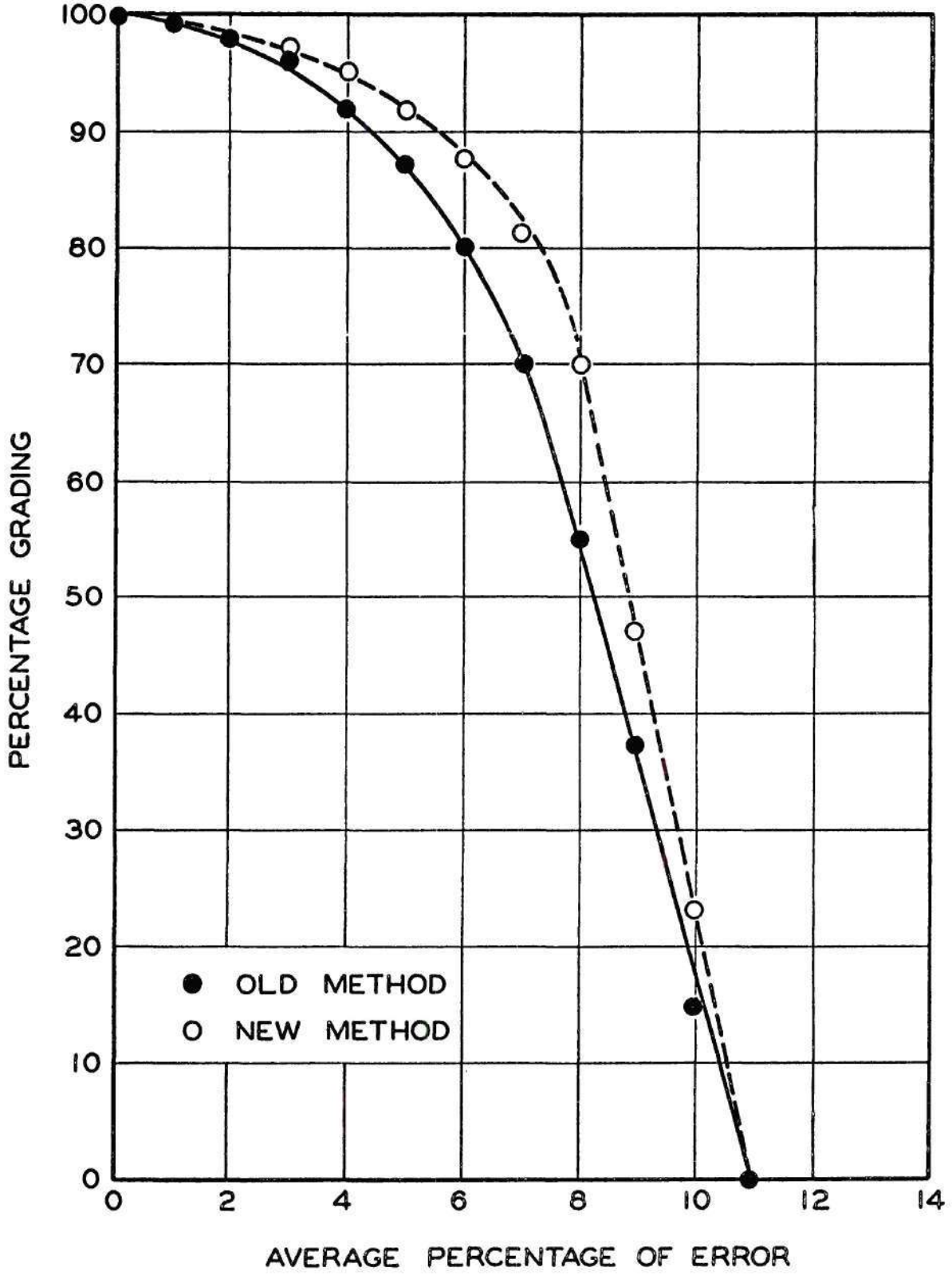


Figure 121. Grading of Quantitative Fiber Analysis

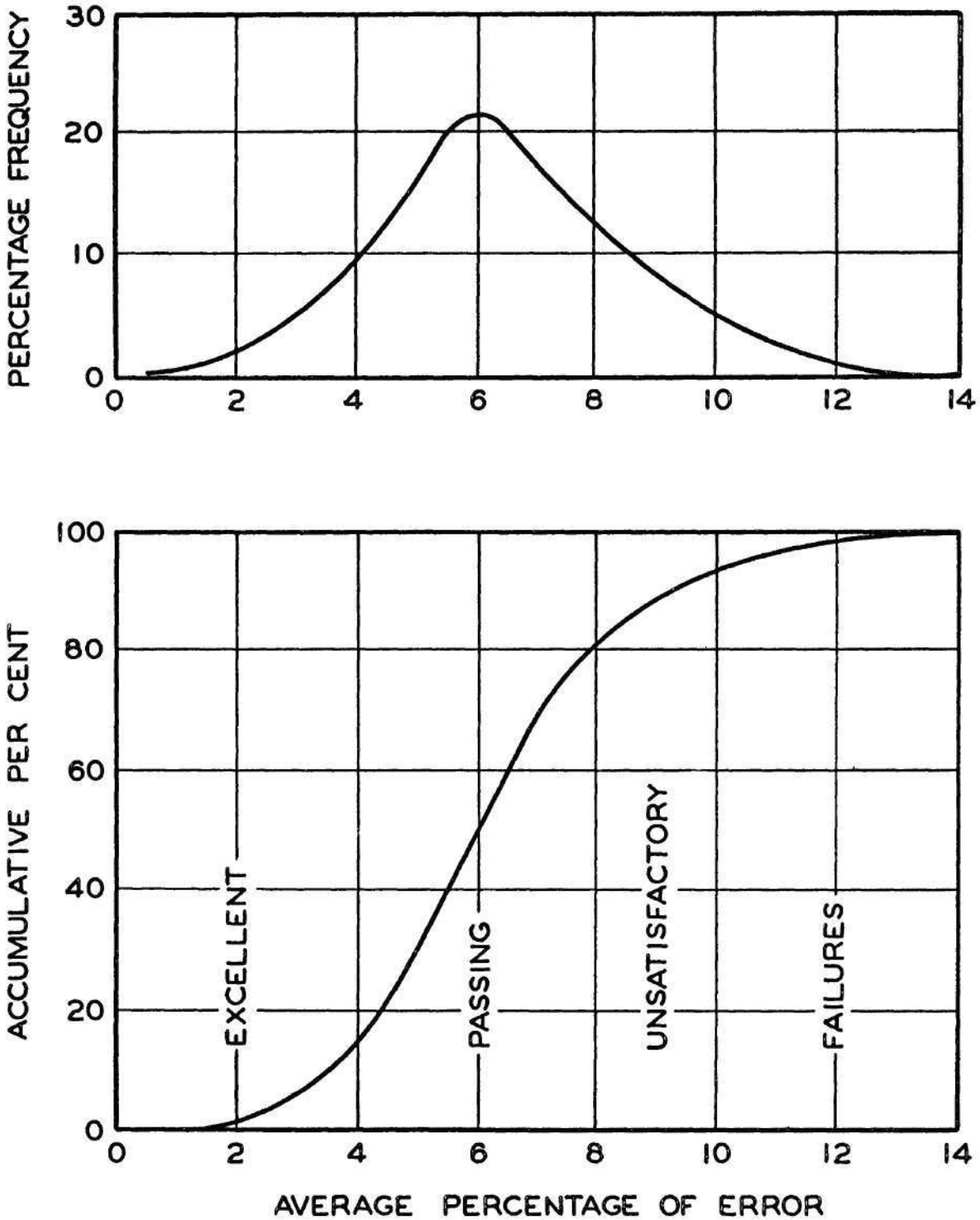


Figure 122. Average Percentage Errors of Fiber Analysis

Example 1

Percent of Analysis	Grading, %	Rating, %
10	99	990
20	98	1960
30	96	2880
20	92	1840
10	87	870
10	80	800
		9340

Rating = 93.40%

Example 2

Percent of Analysis	Grading, %	Rating, %
10	99	990
10	98	980
10	96	960
10	92	920
20	87	1740
10	80	800
10	70	700
10	55	550
5	37	185
5	15	75
		7900

Rating = 79.00%

SUMMARY

Fiber analysis today is much more complicated than it was even 10 or 15 years ago because of the various methods of cooking, bleaching, and purification of the ordinary chemical pulps, and also because of the many new types of high-yield pulps used in paper and paperboard products.

The standard method of pulping should require the use of larger minimum samples.

Disintegration of the sample is a matter of experience and judgment.

There are too many variations in the method of preparing slides. The slides should be prepared in such a way that one-inch squares at the ends of each slide have an even distribution of fibers and that the density is low enough to permit the examination in detail of individual fibers and yet high enough so that a traverse of five lines across the field results in a total count of not less than 200 fibers. Care should be taken that the slides are not too heavy because of error and difficulty in trying to count slides containing 700 to 800 fibers, particularly groundwood.

There is no universal stain and the possibility of developing one is becoming increasingly remote as more and more pulp modifications are devised. The "C" stain is the best general stain and its use by a trained analyst, coupled with a knowledge of fiber morphology, can yield good results with a minimum amount of slide preparation. In certain cases it may be desirable or necessary to use supplementary stains to narrow the identification or to confirm "C" stain borderline judgments. Various miscellaneous stains will be considered in the next chapter.

Analysis of both ends of the slide must be made and the results of each end should agree within two percent; if not, a third and possibly a fourth field must be counted and the average of all taken as the result.

In case of dispute, and in all court cases, the percentage by number and also the percentage by weight should be reported. The weight factors used should be given.

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CHAPTER IX

MISCELLANEOUS STAINS AND REAGENTS

GROUNDWOOD REAGENTS

Stains used to determine the relative amount of groundwood in paper are based primarily on tests for lignin. These reagents are commonly applied directly to the paper, and the relative amount of groundwood is determined by the intensity of the color reaction. It must be kept in mind, however, that the intensity of the color reaction is also influenced by the degree of cooking and the reaction of the chemical pulp present, as in the case of very raw chemical pulp, which will also react with the stain. For the most part, such tests serve the purpose of rapid preliminary screening, and the chief value of a positive reaction is to suggest the possibility of groundwood and the need of further examination.

ANILINE SULFATE (1)

One gram of aniline sulfate is dissolved in 50 ml. of distilled water and the solution is acidified with one drop of concentrated sulfuric acid. The stain produces a yellow color with paper containing a large percentage of groundwood.

PHLOROGLUCINOL AND HYDROCHLORIC ACID (2)

One gram of phloroglucinol is dissolved in 50 ml. of alcohol and 25 ml. of concentrated hydrochloric acid are added. As the solution deteriorates rather rapidly, it should be prepared immediately before use. The reagent colors groundwood carmine red, but also colors other materials in paper red, e.g., Metanil yellow when used as a dye. This reagent may also be applied to fibers on a slide prepared in the usual manner.

p-NITROANILINE (3)

p-Nitroaniline (0.20 g.) is dissolved in 80 ml. of distilled water and 20 mg. of sulfuric acid (sp. gr. 1.767). This stain has the advantage over an alcoholic solution of phloroglucinol and hydrochloric acid in that it may be kept for years. It is also preferable to aniline sulfate which, although stable, gives a yellow color with wood pulp, which is not easily distinguished under artificial light. p-Nitroaniline colors wood pulp orange to brick red, and the color can be seen under any type of illumination. The reagent has the further advantage of forming a color with unbleached chemical pulps. Colors formed are: Mechanical wood pulp, dull orange to orange; unbleached sulfite pulp, very similar to preceding; bleached sulfite pulp, yellow.

If the pulp fibers are first treated with ammonium molybdate solution, however, the stain gives these colors: Mechanical wood pulp, bright reddish

orange; unbleached sulfite pulp, faint dull orange or faint brown; bleached sulfite pulp, colorless.

HYDROCHLORIC ACID-ALCOHOL STAIN (4)

Two pieces of the paper to be examined are dipped in a solution made by mixing 20 ml. of concentrated hydrochloric acid with 80 ml. of alcohol, squeezed between blotters, and placed on the grid over a hot plate to dry. It is important that the papers are placed with the wire side of one sample and the felt side of the other sample next to the grid, because the staining reaction is stronger on the upper side of the samples. This reaction becomes darker with decrease in the content of groundwood in the paper, because it blackens all chemical pulps. The stain is suitable for demonstrating two sidedness of a groundwood sheet and can also be used with coated papers, except in the presence of calcium carbonate.

AMMONIUM CHLORIDE-ALCOHOL STAIN (4)

If calcium carbonate is present in the coating or in the filler of a paper, the following stain can be used: Dissolve 35 g. of ammonium chloride in 100 ml. of distilled water and add 35 ml. of alcohol. This is used in the same way as the hydrochloric acid-alcohol stain.

CONGO BLUE AND COTTON BROWN (5)

Schulze has proposed a mixture of two substantive dyestuffs, Brilliant Congo Blue 2RW and Cotton Brown N, to differentiate groundwood and chemical pulps. One gram of each of the dyes is dissolved in 70 ml. of distilled water by warming on the water bath. The two solutions are stored separately. Immediately before use, a mixture is prepared of equal parts of the two dye solutions and a 6% solution of crystallized sodium sulfate.

A loose lump of pulp about the size of a pea, prepared in the usual manner from a sample of paper, is put into a test tube with 6-8 ml. of the dye mixture, covered, and boiled for 30 seconds over a Bunsen burner. The solution is separated from the fibers by means of a fine sieve. The fibers are washed with 500 ml. of water in a 500-ml. Erlenmeyer flask, again filtered, and diluted to the proper concentration; slides are made in the usual manner. A drop of Canada balsam is applied and a cover glass is put on the slide.

Preparations made in this way give a clearer field than when a zinc chloride-iodine solution is used; crossed fibers are not obscured but remain transparent, and fibrils from chemical or groundwood pulps are distinctly colored according to their origin. Chemical pulps show a clear blue with a faint tendency toward violet; groundwood pulp appears brown.

Substitutes for Congo Blue and Cotton Brown

At least two workers have proposed alternative dyes to be used with the technique of Schulze (5). Leach (113) suggested that equal volumes of 3% Coomassie Brilliant Blue FF 200 and 3% Chlorazol Fast Orange W.S. be used with an equal volume of 6% potassium sulfate as a mordant. The dyes are mixed and allowed to stand for one hour. Mechanical pulp fibers are stained blue-black and chemical pulp fibers red-brown.

Armitage (114) suggested the use of Chlorazol Catechin GR 150 and Chlorazol Azurine G 200. The color reactions are similar to the dyes used by Schulze (5), although the red-brown for mechanical pulp fibers is less reddish and the blue for the chemical pulp fibers is more a slate-blue in the case of the chlorazol dyes.

Armitage (115) also proposed the use of the Lofton-Merritt stain (26) for this purpose, reporting that mechanical pulp fibers were stained intense blue-green. It was pointed out, however, that a mixture of equal volumes of 1% solutions of Malachite green and gossypimine gives better differentiation than the Lofton-Merritt stain, with mechanical pulp fibers staining deep blue-green and chemical pulp fibers rose-red.

SULFANILIC ACID (6)

Noll has described the use of sulfanilic acid in dry form, either as stick or reagent paper, or as a liquid ink. When using the stick form, the paper to be tested is moistened with a drop of water and a line is drawn with the stick across the moistened spot; when groundwood is present, an intense yellow color appears. In the case of the reagent paper, a well-moistened strip is placed between two layers of the paper, the whole being pressed lightly by hand; in the presence of groundwood, the yellow color will appear. A suitable ink is prepared from 20 g. sulfanilic acid, 40 g. urea, 40 g. glycerin, and 900 g. distilled water. If chemically pure reagents are used, the solution will keep well in a glass-stoppered bottle; it is not sensitive to light. It is best to apply it with a glass stylus, although steel pens are, in general, not corroded by the solution. The writing is colorless at first but changes to a more or less intense yellow, depending upon the amount of groundwood present. Five minutes should be allowed after application before a final conclusion is drawn. The advantage of sulfanilic acid is that it does not affect the skin or clothes of the operator when accidentally spilled; it is easy and convenient to handle.

DIFFERENTIATION BETWEEN SOFTWOOD AND HARDWOOD GROUNDWOOD

Methylene Blue (7)

The slide is stained with a solution of aniline sulfate, which colors all the fibers yellow; it is then stained with an aqueous methylene blue solution (1:5000) which does not stain the softwood but stains the previous yellow-stained hardwood fibers a bluish green color.

Mäule Reaction

The application of the Mäule stain reaction for the differentiation of hardwood and softwood mechanical pulps has been evaluated by Spearin and Dressler (8). Mixtures of commercially produced hardwood and softwood groundwoods were treated with the Mäule stain with the observation that, by the use of appropriate weight factors and an empirical correction, either of the components of the mechanical pulp mixture can be estimated to ± 5%. Groundwood bleaching by hypochlorite or by peroxide apparently does not affect the Mäule stain reaction.

The following procedure was used in the evaluation by these authors (8):

Five drops of a 1% potassium permanganate solution were applied to the fiber field on the slide and the slide tilted to spread the solution evenly. The stained area was allowed to remain in contact with the permanganate for 2 to 3 minutes.

The slide was dipped into distilled water to wash the permanganate from the fiber area. If carefully done, no fibers are removed in this rinsing operation.

Two drops of 6N hydrochloric acid were applied to the fiber area and, after a few seconds, the slide was dipped into distilled water to remove the acid.

Two drops of ammonium hydroxide solution (1 part concentrated hydroxide to 5 parts distilled water) were applied, followed by a cover glass. The excess liquid was then drained off and a fiber count made in the customary manner.

DIFFERENTIATION BETWEEN UNBLEACHED AND BLEACHED GROUNDWOOD

Stannous Chloride (9)

As it is practically impossible to wash out all the sulfur dioxide or sulfite used for bleaching groundwood, the residual bleach is detected by boiling some of the pulp with stannous chloride and testing for hydrogen sulfide. Bleached groundwood gives a positive test even after a lapse of several months.

Lofton-Merritt Stain (10)

Graff determined that the Lofton-Merritt stain (see page 259) was useful in distinguishing peroxide-bleached groundwood from unbleached groundwood.

The fibers were prepared on the slide in the standard manner, the stain was added to the fibers on the slide and allowed to stand for two minutes, after which the excess stain was drained off and the fibers were rinsed thoroughly with distilled water. Two or three drops of water were added to the slide, the cover glass was mounted, and the water was drained off. Staining can also be performed directly in a beaker.

According to Graff this stain gives the following color reactions: Unbleached groundwood, deep blue violet to pale violet; commercial peroxide-bleached groundwood, amethyst violet to light Hortense violet. It is advisable to examine known samples before analyzing unknown ones, especially when the colors show such slight differences.

DYES FOR FIBER DIFFERENTIATION

Very early in the history of fiber technology attempts were made to secure differential staining of various types of fibers by the use of dyes. The results have been more or less successful attempts to distinguish unbleached and bleached sulfite, unbleached sulfite and unbleached kraft, and to measure the degree and uniformity of cooking, bleaching, and purification of the fibers. Although many of these methods are at present of limited interest, others are valued and used in many mill laboratories.

BEHREN'S DIFFERENTIATION BETWEEN UNBLEACHED AND BLEACHED SULFITE (11)

A saturated solution of Malachite green in a 2% solution of acetic acid gives the following results on sulfite pulps: Unbleached, blue green to green; semibleached, sky blue; and fully bleached, colorless.

KLEMM'S DIFFERENTIATION BETWEEN UNBLEACHED SULFATE AND BLEACHED SULFITE (12)

The stain is a saturated solution of Malachite green in a 2% solution of acetic acid. A 2% alcoholic solution of rosaniline which is mixed with sulfuric acid until it has taken on a violet tint gives a deep violet red color with unbleached sulfite, a less intense more reddish color with both bleached sulfite and unbleached soda, and only a faint reddish tint or no color with bleached soda. Unbleached soda is stained a clear green color with the Malachite green solution but bleached sulfite is colored faint blue or colorless.

SCHWALBE'S DIFFERENTIATION OF SULFITE AND SULFATE FIBERS (13)

The paper is disintegrated and freed from resins with alcohol-ether; the dried material is covered with 0.05N ferric chloride solution, and heated on the water bath at 60-80°C. until the fibers sink to the bottom of the beaker (about 0.5 hr.). After filtering, the material is washed with warm distilled water, covered with 1% sulfuric acid, 4 to 8 drops of 2% potassium ferrocyanide solution are added, and the whole is heated for 5-10 minutes in a water bath at 60-80°C. The fibers will then appear green or blue. When a few more drops of the potassium ferrocyanide solution and sulfuric acid are added, sulfite fibers become deep blue, unbleached sulfate is colored weak yellow greenish brown, and bleached sulfate is colorless or a very weak blue.

SHAFFER'S DIFFERENTIATION BETWEEN BLEACHED SULFATE AND SULFITE (14)

One gram of sodium carbonate is dissolved in 175 ml. of distilled water and one gram of C.P. brazilin is added to this solution with stirring until the dye is dissolved. Sharper differentiation results if the solution is freshly prepared before use. The pulps must be disintegrated with distilled water, as no alkali or acid can be used. The solution may be applied directly to the sample to be identified or a microscope slide may be dipped into the stain. The excess stain is removed instantly, a few drops of U.S.P. white paraffin oil are placed on the slide, and the excess oil is removed. Bleached sulfite stains wine red and bleached kraft stains a purple color.

CYANINE REAGENT (15)

Herzog has shown that the cyanine reagent can be used to determine the bleachability of a pulp and also to differentiate between sulfite and soda or sulfate pulps. The solution consists of a cold saturated alcoholic solution of cyanine (Quinaldine blue). This solution is diluted somewhat with water and with 1/3 of its volume of glycerin. The stain is used warm except when it is desired to show the presence of silk sericin.

a. Bleachability or degree of cooking of softwood pulps. The cyanine-glycerin solution stains as follows: Tracheids and ray cells stain a definite

blue; if lignin is left in the tracheids, they stain an intense violet blue. Fully bleached cellulose is colorless.

b. Differentiation between sulfite and sulfate pulps. In unbleached sulfite fibers the bordered pits are colored an intense blue but in sulfate fibers this is seldom the case; if a color results, it is very faint. At the same time, the ray cells in sulfite pulps are colored dark blue because of the resin present. Bleached fibers in both pulps are colorless, except that the ray cells in the bleached sulfite are colored an intense blue violet. Many prefer this test to Klemm's Sudan IV, because the latter gives only a weak orange color and does not show the lignification of the fibers.

The sericin is colored dark blue, but the fibroin remains colorless, when silk fibers or fabrics are treated with cold cyanine solution. When viscose fibers are treated with warm cyanine-glycerin solution, impurities are colored dark blue.

NOLL'S COLOR DIFFERENTIATIONS

Noll (16) has developed a stain which may be used for the separation of mechanical, unbleached and bleached chemical (sulfonated) pulps. It contains 0.1 g. basic color, 25 ml. methyl glycol, 25 ml. glycerin and 25 ml. of 4% aniline sulfate solution. After the mixture of methyl glycol, glycerin and aniline sulfate solution is prepared the dye is dissolved by heating and shaking (boiling must be avoided). When cool, the solution is filtered and is ready to use. The reagent is stable. The color contrast between groundwood (yellow) and unbleached pulp (blue) is very good with methylene blue, prune pure (Gallo blue E), and Celestine blue B, and good with crystal violet. The bleached pulp remains colorless.

BRIGHT'S STAIN

In 1917, C.G. Bright developed a stain to differentiate unbleached and bleached sulfite pulp (17). The original method was very difficult because of the critical temperatures employed, and was modified (18). The method has many drawbacks and was criticized by several workers (19, 20).

In 1929, Graff and Calkin performed a series of experiments with this stain in the following manner: 1.5 g. of disintegrated pulp were squeezed dry and dyed in a beaker with the ferric ferricyanide solution for 20 minutes at room temperature, washed twice with 500 ml. of distilled water, dyed with benzo-purpurine 4B in a beaker for 20 minutes at room temperature, and again washed twice with 500 ml. of distilled water. The dyed fibers were made into test tube suspensions and mounted in water on the microscope slide.

The color reactions of unbleached fibers exhibited wide variation-blue, gray blue, blue with red haze or markings, red with blue haze or markings, and various tints of red.

Later, Kantrowitz and Simmons (21) suggested another modification of the Bright stain, which is now included in TAPPI method T 401 m-60.

Their stain modification is prepared as follows:

Solution A: 2.7 g. of ferric chloride in 100 ml. of distilled water;
Solution B: 3.29 g. of potassium ferricyanide in 100 ml. of distilled water;
Solution C: 0.5 g. of crude benzopurpurine 4B in 100 ml. of 50% alcohol (dissolved hot).

Equal parts of A and B are mixed, a few drops are placed on the slide, and allowed to stand for one minute; the excess is removed, the slide is rinsed in distilled water, dried and stained with Solution C. After staining for two minutes, the excess dye is removed, the slide is rinsed, and a cover glass is added.

The color reaction of the individual fibers of an unbleached pulp, when stained with any of the Bright stain methods, varies proportional to the degree of cooking from blue to gray blue, blue red, red blue, and red.

To check these results, the different color reactions of the Bright stain were assigned arbitrary values: Blue, 20% cooked; gray blue, 40%; blue red, 60%; red blue, 80%; and red, 100%.

Four unbleached sulfite pulps with different permanganate numbers were stained by the modified Bright stain method (22); the percentage by number of the fibers in the five main color groups for each of the pulps was determined; these percentages were multiplied by the arbitrary numerical value of the degree of cook; and the sum of the calculated values was determined. Two sets of counts were made for each pulp to indicate the accuracy by which the values may be duplicated. An illustration of such a calculation is given in Table XXVI.

It was shown (22) that there is a good check between the counts of the fibers showing different color reactions for each sample and that the average cooking value and the percentage by number of the pure red fibers increase in indirect proportion to the permanganate number of an unbleached sulfite pulp, i.e., the lower the permanganate number of an unbleached sulfite pulp, the larger will be the number by percentage of red fibers recorded as bleached.

These results demonstrate that the Bright stain cannot be used as a standard for the determination of the percentage of unbleached and bleached pulps in a paper furnish but that it is a quick method for determining the approximate degree of cooking and uniformity of cook of a pulp.

This stain may be applied either to fibers on the slide, or 1.5 g. of the fibers may be stained in 50 ml. of the solution in a beaker. In either case, mix equal parts of Solutions A and B just before using; apply for one minute at room temperature, thoroughly wash the stain mixture from the fibers, and then stain them for two minutes with Solution C. After staining, again wash thoroughly before observation.

TABLE XXVI

DETERMINATION OF UNIFORMITY OF COOKING OF PULP

Permanganate Number-9.60

Color	Cooking Value, a	Frequency	Percentage by Number, b	a x b, %
Blue	20	251	83	16.60
Blue gray	40	38	12	4.80
Blue red	60	9	3	1.80
Red blue	80	5	2	1.60
Red	100	0	0	0.00
Total		303	100	24.80

COOKING OR BLEACHABILITY STAIN

If unbleached pulp is dyed by a standard method, first with Malachite green and then with Congo red, and microscope slides are prepared, it will be found that some fibers are dyed pure green, some lighter green with red markings, others red with green markings, and some pure red (23). The relative proportions of the differently colored fibers in the pulp depend upon the degree to which the pulp has been cooked (11). The more red fibers with green markings or pure red fibers present, the more the pulp has been cooked; the more green fibers with red markings or pure green fibers, the less the pulp has been cooked (24).

To calculate a value for degree of cooking and also to determine the uniformity of the cook the various colors have been assigned arbitrary values, in a manner similar to that used with the Bright stain (22).

Variations of green	10% cooked
Blue with green markings	20%
Red with strong green markings	40%
Red with faint tint of green	60%
Lightly broken red	80%
Pure red	100%

By this method, not only the degree of cooking of the pulp, but also the uniformity of the cook can be determined. The process by which the pulp is made and the kind of fibers used have no bearing on the results.

A method for determining the degree of cooking of a pulp, which appears to be better and more accurate, involves treatment of enough pulp to form a

sheet in a Büchner funnel. The sheet is dried and its color is compared with a standard scale which has been given arbitrary evaluations from 30 to 124 1/2 +.

The following solutions are used in this procedure (22):

Acetic acid – 10%;
Malachite green – 0.5%;
Ammonium carbonate – 10 g. of ammonium carbonate in 90 ml. of distilled water;
Congo red – 0.1%;
Calcium chloride – 30 g. of calcium chloride are dissolved in 2500 ml. of distilled water; 1.25 ml. of this solution are added to every liter of distilled water for washing the pulp. This is a 0.0015% solution.

Standardized Procedure

A representative sample of pulp is mixed with water in an Erlenmeyer flask and thoroughly disintegrated by shaking the flask vigorously. The pulp suspension is then filtered on a "varnitized" filter fabric in a Büchner funnel. Ten grams of this moist sample are transferred to a 250-ml. beaker and thoroughly mixed with 200 ml. of 10% acetic acid. The pulp is filtered as before, and mixed in a beaker with 200 ml. of 0.1% solution of Malachite green at 70°C. The beaker is placed on the steam bath maintained at 70°C., for three minutes, during which the pulp suspension must be stirred constantly. Again the pulp is filtered as before and the excess liquid squeezed out. Then the sample is placed in a 500-ml. Erlenmeyer flask and washed with five changes of 500 ml. of 0.0015% calcium chloride solution.

The washed pulp is again transferred to the 250-ml. beaker and thoroughly mixed with 200 ml. of 10% ammonium carbonate solution. The pulp is filtered off and mixed in a beaker with 200 ml. of 0.05% Congo red which is at 70°C. This is heated on the steam bath at 70°C. for three minutes, with constant stirring of the pulp suspension. The pulp is again filtered and the excess liquid squeezed out. The sample is again placed in a 500-ml. Erlenmeyer flask and washed with five changes of 500 ml. of 0.0015% calcium chloride solution.

The washed pulp is drained on a piece of felt in a 5.5-inch Büchner funnel. The drained sheets with their supporting felts are placed one on top of the other and put in a press to squeeze out the remaining water. The sheets are separated from the felts, squeezed with a rubber roller on a lacquered plate, and placed in a constant temperature drier at 70°C., which has been selected as the standard temperature for drying the dyed sheets.

When the sheets are thoroughly dry, a strip equal to the width between the standard color samples is cut from each and matched as closely as possible with the cooking stain scale (24) in a specially constructed reading box illuminated with daylight fluorescent light. The cooking stain standards are arranged on a long strip of canvas-backed drawing paper and fastened on a wheel suspended in a box. The wheel can be rotated on its axis, and the strips to be measured can be compared with the standard through an opening in the box. The lamp is mounted on top of the box (22).

The cooking stain values are much lower when the dyed pulp is washed with distilled water than when tap water is used. The distilled water washes out very little of the green dye in the pulp but it removes nearly all of the red dye. A series of experiments showed that the observed differences in the cooking stain values were in proportion to the relative hardness of the water. As a result of these studies, a 0.0015% calcium chloride solution has been adopted as the standard for washing the dyed pulps (22).

A single test can be made in half an hour and six tests in one and a half hours. One person can easily make 18 to 22 tests in a day.

Correlations

The four sulfite pulps used to demonstrate the inaccuracy of the Bright stain for determining the percentage of unbleached and bleached pulp were dyed in the above-described manner. It was found that as the lignin content of the pulp decreased, the cooking stain value increased, but the relationship is not linear (22).

Microscope slides were also prepared from these four dyed pulps and the percentage by number of the fibers in the six main color groups for each pulp was determined; the percentages were multiplied by the arbitrary value of the degree of cooking given to each color reaction group, and the sum of the calculated values determined. The data shown in Table XXVII were obtained for the same pulp as used in the studies with the Bright stain, illustrated in Table XXVI.

In this case, also, there is a good check between the counts of the fibers showing different color reactions for each sample; the average cooking value, as with the Bright stain, and the percentage of pure red fibers increase in indirect proportion to the permanganate number of the pulp; this shows that the stain cannot be used for the determination of unbleached and bleached pulps.

TABLE XXVII

DETERMINATION OF UNIFORMITY OF COOKING OF PULP BY COOKING STAIN METHOD

Permanganate Number-9.60

Color	Cooking Value, a	Frequency	Percentage by Number, b	a x b %
Green	10	58	28	2.8
Blue green	20	83	39	7.8
Green red	40	63	30	12.0
Red green	60	4	2	1.2
Broken red	80	3	1	0.8
Red	100	0	0	0.0
Total		211		24.6

BLEACH STAIN

Wasicky (25) claimed that papers made of pure soda wood pulps are not dyed, whereas sulfite papers are stained a deep violet when treated with Gentian violet. A thorough investigation of this stain revealed that sulfite pulps are dyed various shades of blue violet in proportion to the degree of bleaching of the pulp (22). The colors ranged from dark blue violet for the very lightly bleached pulps to a faint tint for the highly bleached and purified pulps. Soda and sulfate pulps, instead of being colorless, also varied in strength of color in proportion to their purification, but the color reaction was very heavily broken blue violet.

The stain cannot be used for the differentiation of sulfite and soda, particularly because the individual fibers show no color under the microscope. By modifying the staining method and by making sheets of the dyed pulps, it was found that the stain gave an excellent determination of the extent to which the pulp had been bleached, or the relative purity of the bleached pulp (24). The different color reactions are given arbitrary numerical values in proportion to the strength of the color, so that the stronger the color, the lower the numerical evaluation, and the fainter the color, the higher the evaluation.

This stain was very valuable for evaluating the degree of bleaching of a pulp during the time when color wheels were used for the determination of the brightness of the pulp. It is also an excellent method for the determination of the dyeing quality of rayons and other textiles.

Standard Method

A representative sample is thoroughly disintegrated and drained on a Büchner funnel covered with a "varnitized" filter fabric. Ten grams of this pulp are put into a 250-ml. beaker and mixed with 200 ml. of a 0.2% solution of Gentian violet for five minutes, drained, and returned to the beaker; 200 ml. of alcohol are added and the mixture is stirred for two minutes, drained, and returned to the beaker. After adding 200 ml. of 85% alcohol containing 0.5% hydrochloric acid, the mixture is stirred well for two minutes, the pulp drained, and returned to the beaker. The pulp is given two six-minute rinsings in 85% alcohol, drained, and given two rinsings with distilled water, using a 500-ml. Erlenmeyer flask, and sheets are made the same as for the cooking stain. These operations are performed at room temperature. The sheets are dried in an oven at 70°C.

LOFTON-MERRITT STAIN

In 1921, Lofton and Merritt (26) described a method for differentiating and estimating unbleached sulfite and sulfate pulps in paper. The stain consisted of one part of a 2% solution of Malachite green and two parts of a 1% solution of basic fuchsin or magenta (General Dyestuffs Magenta AB powder). The solutions are kept separately in tightly stoppered bottles, and are mixed only when wanted for use.

Several fibers are placed on a microscope slide, and dried by the use of hard filter paper or blotting paper. Two or three drops of the compound stain are then placed on the fibers and allowed to remain for two minutes, during which time the fibers are teased apart and moved about in the stain on the slide. This teasing is necessary in order that the stain may have an equal opportunity to act on all the fibers. After two minutes the excess stain is removed with three or four thicknesses of hard filter paper, and the fibers are treated with three or four drops of very dilute hydrochloric acid, made by adding 1 ml. of concentrated acid (sp. gr. 1.19) to one liter of distilled water. The dilute acid is allowed to remain on the slide for 10-30 seconds, during which time the fibers are teased and moved around rapidly. Following this, the excess acid solution is removed with filter paper, three or four drops of distilled water are applied, the fibers are quickly teased apart, and the water absorbed with filter paper. If all of the excess stain has been removed from the slide, a drop or two of water may be added, the fibers spread about the slide, and a cover glass placed over them. However, if too much stain remains on the slide, it will be necessary to rinse again with distilled water before applying the cover glass. After the cover glass has been placed in position, the fibers are ready for examination under the microscope.

Wisbar's Modification

Wisbar (27) modified the technique of Lofton and Merritt by using the same amount of mixed dyes but added the hydrochloric acid directly to the mixture. The dye mixture was placed in a beaker, a small lump of the disintegrated fibers added, and the suspension cooked for one to two minutes. By this treatment the sulfite fiber is stained red violet and the kraft fiber greenish blue.

Although both methods, as a rule, give good differentiation between the two fibers, the quantitative analysis may differ at times from the actual amounts present. A larger amount of sulfite was found by Lofton and Merritt's method than by dyeing the fibers directly (28). From this one might believe that dyeing directly on the slide would give too much red to the kraft fibers and dyeing in the beaker would give too much blue to the sulfite fibers, but three experiments did not show any noticeable difference between the two methods.

Graff's Stain

In 1927, Abramsen and Graff (unpublished) did a series of tests to determine the accuracy of analysis of mixtures of unbleached sulfite and sulfate, cooked to different degrees, and also to determine the influence of bleached sulfite and kraft on the analysis.

Solution A: Five grams of fuchsin red dissolved in 100 ml. of alcohol and diluted with 400 ml. of distilled water.

Solution B: Ten grams of Malachite green dissolved in 100 ml. of alcohol and diluted with 400 ml. of distilled water.

Thirty milliliters of the fuchsin red solution, 15 ml. of the Malachite green solution, and 0.09 ml. of hydrochloric acid were mixed, 1.5 g. of disintegrated pulp (squeezed dry) added, and the suspension was stirred at room temperature for two minutes. The pulp was then strained, squeezed dry between the fingers, washed once with distilled water in a 500-ml. Erlenmeyer flask, strained, and diluted to proper consistency. A slightly better result was obtained if the dyeing was carried out on a steam bath at 70°C. for two minutes (22).

Color reactions: Rag filter paper, colorless; bleached alpha, mostly colorless; unbleached alpha, colorless, a few purple fibers; bleached sulfite, very pale pink; unbleached sulfite, purple to colorless; bleached sulfate, pale pink; unbleached sulfate, grayish blue violet; bleached soda, pale purple; unbleached soda, very pale purple; and groundwood, deep blue violet.

The color reactions show that, ordinarily, unbleached kraft could be determined easily, but the presence of bleached sulfite and sulfate made the determination of the amount of unbleached sulfite somewhat difficult. Because the literature has often referred to the use of this stain for the differentiation of unbleached and bleached sulfite, experiments were made to determine to what extent the degree of cooking of the unbleached sulfite would influence the accuracy of the determination of bleached and unbleached sulfite by this staining method. It was concluded that the degree of cooking of the unbleached sulfite does not interfere with the fairly accurate calculation of mixtures of unbleached and fully bleached sulfite pulps (22).

On the other hand, the degree of bleaching is a very important factor in the correct analysis of such mixtures according to the results of analyses of 50-50 mixtures of unbleached sulfite and sulfite bleached to varying degrees; the stain can be used only when the pulp is fully bleached.

Further investigations showed that the relative amount of strong, medium-colored, faint, and colorless fibers were in proportion to the degree of cooking, bleaching, and purification of the fibers (22). This suggested that the different tints should be given arbitrary values. If the percentage by number of each of the differently colored fibers were determined, multiplied by its given arbitrary value, and the resulting figures added together, one should obtain a general factor which would be relatively small for a pulp with a large number of dark-colored fibers and relatively high for a pulp with a high percentage of colorless fibers (if the colorless fibers are given an arbitrary value of 100, on the top of the scale). Since this number increases with the supposed refinement of the pulp, it has been proposed that it be called the "pulp purity factor" (22).

The preceding work shows that, although the Lofton-Merritt stain differentiates between ordinary unbleached sulfite and sulfate, it does not distinguish among unbleached sulfite, the more refined unbleached sulfates, hardwood kraft and bleached kraft. This stain is also unsatisfactory for use in determining unbleached and bleached sulfite, unless the sulfite has been fully bleached and even then a certain amount of error is unavoidable, because, as is shown by the purity factor determinations, unbleached sulfites have a certain amount of lightly colored or uncolored fibers, depending upon the degree of cooking of the pulp. The "C" stain, on the other hand, gives very

definite differentiation, not only between softwood and hardwood unbleached kraft and sulfite, but also between unbleached and bleached kraft.

KLEMM'S METHOD (29)

Certain extraneous components, particularly in the ray parenchyma cells, survive the sulfite pulping process but are usually removed in alkaline pulping. These residues are found even in bleached sulfite pulps. They exist as aggregates or chains of small spherical elements. Their staining capacity depends on the presence of resin, hence, care must be taken not to dissolve the resin by caustic treatment. With an aqueous-alcoholic solution (1:3) of Sudan IV (saturated) with a little glycerin, these residues are stained red; with zinc chloride-iodine, yellow. Sulfite fibers show a characteristic venation in zinc chloride-iodine stain.

Further differentiation between sulfite and sulfate is made with a rosaniline sulfate solution, prepared as follows: One-quarter gram of the crystalline sulfate is added to 50 ml. of boiling water and 50 ml. of warm water are added, followed by 2 ml. of alcohol. After standing for some time, the solution is filtered through asbestos. Finally, 14 drops of 0.1N sulfuric acid are added.

A solution of methylene blue (0.1%) in warm water may also be used.

When these solutions are used with unbleached sulfite fibers, the pit markings are stained red or blue. Sulfate and bleached sulfite do not show such markings.

GREEN AND YORSTON STAIN

A stain which is very useful for the detection of unbleached sulfite fibers is that devised by Green and Yorston (30, 116, 117). Dissolve 15 mg. of p, p'-azodimethylaniline in 100 ml. of glacial acetic acid. After solution is complete, add 300 ml. of distilled water, slowly, with agitation.

Flood the fiber field with the stain, pour off after two or three minutes and replace with fresh stain. After one to two minutes, apply a cover glass and remove the excess stain. Do not overdry the slides.

Fibers of coniferous unbleached sulfite pulp of news grade, or equivalent chlorine number, are stained strongly red. With well-cooked pulps, only the bordered pits are strongly stained and the fiber wall may be only a light pink. Hardwood unbleached sulfite pulps are generally lightly stained. This stain also colors hardwood unbleached neutral sulfite semichemical pulps and may be used to differentiate these and kraft semichemical pulps.

DU PONT STAINS

The five stains to be described and their methods of use are claimed to provide a clear differentiation among all the common papermaking fibers in all possible combinations (31).

The general stain may be used to identify groundwood, rag, and hardwood chemical pulps, and to establish the presence of, but not differentiate, coniferous wood chemical pulps. Five drops of a stain made of 50 g. of zinc chloride and 15 g. of anhydrous calcium chloride made up to 100 ml. with distilled water (Chloride Stain No. 3) are added to the slide and spread evenly. After 20 seconds, add one drop of stain made by carefully mixing 6 g. of potassium iodide and 1.5 g. of crystalline iodine in 100 ml. of distilled water (Modified Herzberg Stain No. 2), and mix by tilting the slide. After one minute from the time the iodine was added, drain the slide and drop on the cover glass.

The V-stain is used to determine if hardwood and coniferous wood chemical pulps have been bleached. Add six drops of stain made by dissolving 5 g. of potassium ferricyanide in 50 ml. of distilled water and 50 ml. of alcohol (Ferricyanide Stain No. 5), add three drops of stain made by dissolving 5 g. of ferric chloride in 100 ml. of distilled water (Ferric Chloride Stain No. 6) and mix by tilting the slide. After one minute, wash lightly and blot. Add a few drops of stain made by dissolving 5 g. of Du Pont Pontamine Bordeaux B in 100 ml. of distilled water (Bordeaux Stain No. 7). After one minute, wash and blot dry. Add one small drop of a solution of 50 ml. of saturated sodium chloride solution in 50 ml. of glycerin and drop on the cover glass.

The W-stain is used to determine whether unbleached coniferous pulp is sulfite or kraft. Add a few drops of stain made by dissolving 2 g. of basic orange dye in 50 ml. of distilled water and 50 ml. of alcohol (W-basic orange Stain No. 8). After 30 seconds, wash and blot. Then add a few drops of stain made by dissolving 0.75 g. Du Pont brilliant green crystals in 25.5 ml. of alcohol, 11.0 ml. of distilled water, and 62.5 ml. of the basic orange stain. After 30 seconds, wash and blot. Finally, add one small drop of the salt-glycerin solution described earlier and mount the cover glass.

The Y-iodine stain is used to differentiate fully bleached kraft from bleached sulfite. Add a few drops of stain made by mixing 20 ml. of distilled water, 40 ml. of alcohol, and 40 ml. of the W-basic orange Stain No. 8 described above. After 30 seconds, wash and blot. Add a few drops of Special Y-iodine stain, prepared by mixing 1 ml. of alcohol, 2 ml. of Chloride Stain No. 3, 3 ml. of Herzberg iodine stain (100 ml. of distilled water, 2 g. of potassium iodide, and 2 g. of crystalline iodine); and 4 ml. of saturated sodium chloride solution. Blot after one minute. Add one drop of Chloride Stain No. 3 and drop on the cover glass. The Special Y-iodine Stain must be prepared fresh.

The X-stain is used to differentiate some high partially bleached krafts from bleached sulfite pulps. Add a few drops of stain made by dissolving 1.5 g. Du Pont brilliant green crystals in 70 ml. of alcohol and 30 ml. of distilled water. After 30 seconds, wash and blot. Add a few drops of Modified Herzberg Stain No. 2. Blot after 30 seconds Finally add a drop of Chloride Stain No. 3, and drop on the cover glass.

Some of these stains are much more useful than others. The W-stain, the V-stain, and the Y-iodine stain have been of particular help.

SIMONS' STAIN (32)

The purpose of Simons' stain is to indicate the degree of mechanical treatment that has taken place in the stock preparation.

The following two solutions are used in mixture:

Solution A: 1.0 g. of a direct blue dye (Color Index No. 518) dissolved in 100 ml. of distilled water.

Solution B: 1.0 g. of a direct orange dye (Color Index No. 621) dissolved in 100 ml. of distilled water.

For the staining of most wood pulps, a solution containing equal parts of the two dyes is satisfactory; for rag stocks, better results are obtained if the orange is in slight excess. The total strength of the staining solution, adding both dyes together, should be about 1%. Most workers will vary the ratio to suit their own ideas of desirable contrast.

Dye mixtures should be made up in approximately 50-ml. volumes, using an accurate graduate or pipet. Use regular 50-ml. dropping bottles and prepare three mixtures of the combined stain, namely Solution A to Solution B:- 55:45, 50:50, and 45:55, by volume.

Six to eight drops of the combined staining solution are added to the fiber field on the slide. Place the slide with the stain on a hot plate at about 70°C. Allow the staining solution to evaporate to dryness, which should be a matter of 5 to 10 minutes. Wash, either in running water or by dipping, or by any convenient method that does not dislodge the fibers. Tap water, if not too hard, is satisfactory. Dry and examine – far more detail can be observed if no mounting material is used. Unbeaten fibers are stained blue. Beaten fibers are stained varying mixtures of orange and blue depending upon the degree of beating. Any fibrils, fiber debris, crushed and bruised spots, and broomed ends, will stain orange. The orange stain preferentially colors any part of the fiber that has been broken down or is mechanically damaged.

This stain has also proved helpful in the examination of cambial and other immature plant tissues. It has also proved useful in the determination of density differences in cellulose fibers (118).

KARMINAZUROL

In 1957, E. Merck AG of Darmstadt, West Germany, announced the availability of Karminazurol nach Kühnel, a new color reagent for the differentiation and estimation of papermaking fibers (33). It is said to serve both for consideration of the fiber mixture under the microscope and also for the preparation of permanent slides.

A small amount (about pea-size) of disintegrated pulp which has been squeezed dry between the fingers is placed in a beaker and covered with the stain (2-3 ml.); this mixture is boiled briefly with shaking two or three times. After filtering, wash on the filter with running tap water. Place some fibers on a microscope slide for examination.

This stain is said to color groundwood blue, unbleached and bleached sulfite bright red, soda or sulfate pulp lilac to violet, and semichemical pulp violet blue to deep dark blue violet; strongly bleached semichemical pulps may be colored red like sulfite pulp (119).

CHLORAZOL BLACK E (34)

A hot aqueous solution of chlorazol black E stains both bleached and unbleached fibers; more important, it also stains fibrils, fibril bundles, primary wall "skins," and other particles of fiber debris, which are stained weakly or not at all by safranin or benzopurpurin. It is almost impossible to overstain and it is not completely removed by normal washing techniques. Because it produces on fibers tones ranging from gray-black through various lighter gray tones, it permits photomicrography without the use of contrast filters. Permanent preparations stained this way have great permanence.

NSSC STAINS

A staining technique to separate unbleached NSSC fibers from other types of fibers involves the use of a solution of 2.0 g. of p-nitroaniline in 100 ml. of 3N HCl, and Herzberg stain. The former solution is applied to the fibers, the excess is removed, and Herzberg stain is applied. An orange color is produced on bleached or unbleached groundwood, a brownish-green color on unbleached NSSC fibers, and a color varying from blue to deep purple (depending upon the type of fiber and type of cook) on chemical pulps and bleached NSSC hardwood fibers (120; see also 117).

The Mäule color test (p. 251) has been applied for the quantitative determination of hardwood NSSC pulp and screenings of softwood chemical pulp. It may also be used to differentiate between hardwood NSSC pulp and full chemical wood pulps (121).

COLD SODA STAIN

The FPL at Madison reports a simple staining treatment to differentiate clearly between cold soda and groundwood pulps under the microscope. When treated with Calcozine Red 6G Ex, cold soda pulps fluoresce a bright orange color and groundwood pulps yellow-green (122).

SPECIAL STAINS AND REAGENTS FOR IDENTIFICATION OF NONWOODY FIBERS

The morphological characteristics as well as some of the chemical reactions of certain nonwoody fibers have been considered in detail in Chapter Six, to which the reader is referred for supplementary information. It is also suggested that various publications on the identification of textiles and related fibers be consulted (35-39, 123, 124).

SELECTIVE STAINS

A simplified scheme of textile fiber classification is outlined in Table XXVIII. In Test 4 of this table, reference is made to Texchrome, one of the many acid and dye combinations which have been developed to simplify the identification of textile fibers. As some of these stains can also be used for the identification of coating and sizing materials, films, and as a rapid method for differential staining of cross sections of plant fibers, a short description is given for some of them.

Picrocarmine K (40)

Two grams of pure carminic acid are dissolved in water and an excess of ammonia is added, changing the bright red color to blue. The solution is heated until the odor of ammonia has disappeared. Then add 15 ml. of 3% picric acid solution which has been neutralized with ammonia. Acidify with dilute hydrochloric acid and dilute to 100 ml.

Animal fibers (degummed silk, wool, Tussah, etc.) are colored yellow, while plant fibers (cotton, flax, hemp, jute, cuprammonium viscose and nitrate rayon) are colored red by the carmine dye. Raw silk gives a deep red brown and acetate rayon a green yellow color. Undyed fibers should be used in the test.

TABLE XXVIII

SIMPLIFIED SCHEME OF TEXTILE FIBER CLASSIFICATION

Test 1 – Apply Flame

Unaffected	Burns		
	- with melting or formation of a bead	- with strong odor and formation of bead	- with light ash-no melting and no bead
Group 1	Group 2	Group 3	Group 4
Glass fiber	Acetate rayon	Wool	Cotton
Asbestos	Nylon	Silk	Linen
Certain fire-proofed fabrics	Vinyon	Hair	Ramie
	Velon	Rubber (natural and synthetic)	Cordage fibers
	Permalon	Casein fibers (Aralac)	Other paper-making fibers
	Other chemical synthetics	Soybean fibers	Viscose rayon
	Also Group 3		Bemberg rayon

Test 2 – Apply Acetone

Dissolves or softens	Unaffected
Acetate rayon	Remainder of Groups 2 and 3
Vinyon	Also Group 4

Test 3 – Apply 90% phenol

Dissolves or softens	Unaffected
Acetate rayon	Remainder of Groups 2 and 3
Nylon	Also Group 4

Test 2 – Abridged Method
Apply combination Acetone – Phenol Reagent

Dissolves or softens	Unaffected
Acetate rayon	Remainder of Groups 2 and 3
Vinyon	Also Group 4
Nylon	

Test 4 – Dye fresh sample with Texchrome (strip colored samples first)

Colored deep blue	Colored red
Bemberg rayon	Viscose rayon

Other colors from these dyeings will confirm tests 1, 2 and 3 – see Texchrome color chart

Neocarmine W, B, and MS (41)

Extract the sample with alcohol for at least one minute and wash thoroughly before treatment with the Neocarmine solution.* It is advisable, although not necessary, to remove size, etc., by boiling with specific agents or soap previous to the staining.

In the case of colored fabrics, the dyestuff must be removed before the test is made. It is impossible to find a solution capable of stripping all coloring bodies; therefore, because of the differences in constitution, various methods may have to be tried. Some dyes may be removed by boiling the material in water and others by treating the sample with 1% cold or warm solution of acid or alkali. Hydrosulfite will serve in many cases. Mixtures of hydrosulfite and Leucotrop, with a very small addition of anthraquinone and a few drops of sodium hydroxide, can often be used with success. Many indanthrene dyestuffs, which have been given a preliminary treatment with hydrosulfite, dissolve on subsequent extraction with hot pyridine, preferably in the Soxhlet apparatus. After removal of the colors, the test piece must be washed thoroughly. Sulfur and certain other dyes can be removed easily in most cases by warming with dilute chlorine water. In this case, after the disappearance of the coloring material, the sample is placed in antichlor (sodium thiosulfate) or in sulfurous acid, and thoroughly washed before treatment with the reagent.

According to Schafer's method (42), first moisten the sample with alcohol and immerse in Neocarmine W at room temperature for 4 to 5 minutes, then 2 minutes in running water, then in 3 to 4% ammonia water and finally rinse. Schafer also suggests that the sample, after being stained with Neocarmine W, be immersed for 2 to 3 minutes in a sodium plumbite solution at 80-90°C., and then rinsed and dried.

A full description for using the stain and a color chart showing the reaction on the different types of fibers is obtainable with the stain. This stain has given excellent results in differential staining of plant tissues.

Colotex B (43)

Dyed samples are stripped of all color, taking particular care that all the stripping agent is completely washed out. The fibers are immersed in Colotex B** for three to five minutes, as specified. Remove from the dye, wash in running water until clean, pass through water containing a few drops of ammonia, rinse in running water and dry.

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* Neocarmine reagents may be purchased from F. F. Kraus Company, 136 Liberty St., New York, N. Y. 10006.

** Colotex B is distributed by Neuberg Chemical Corp., 441 Lexington Ave., New York, N. Y. 10017.

The colors shown by common fibers are: Acetate, lemon yellow; kapok, dull greenish yellow; coir, dull yellow brown; cotton (bleached), dull violet; cotton (bleached and mercerized), blue violet; cotton (unbleached), dull lavender; cuprammonium, reddish blue; hemp, dull light brown; jute, light reddish brown; linen (raw), bluish mauve; nitrate, dull red violet; nylon, dull reddish yellow; silk (degummed), light reddish tan; silk (raw), olive drab to black; silk, Tussah (unbleached), golden yellow; silk, Tussah (bleached), yellowish orange; viscose, lilac; wool (chlorinated), dull brownish orange; wool (unchlorinated); deep maize.

Texchrome (44)

This is a combination and modification of the two Hahn Stains A and B, and consists of the following mixture: Acid fuchsin (Color Index No. 692), 6 g.; picric acid, 10 g.; tannic acid, 10 g.; National Soluble Blue 2B Extra (Color Index No. 707), 5 g.*

The dyes may be ground and dissolved together, or dissolved separately in any order and diluted to one liter. Although the overall concentration may not be very critical, these ratios of components appear to give the best differentiation. The dye mixture can be dissolved readily only in hot water, but the solution may be used hot or cold. Momentary immersion in the hot solution is sufficient, but commonly something over two minutes is allowed for cold dyeing. A thorough rinsing in water completes the test. Some dyed textiles may be identified without previous bleaching. The fibers are treated as usual and then rinsed. When pressed wet (after rinsing) between white absorbent papers, a dye mixture characteristic of the color which would have been shown by the undyed fibers is transferred to the papers.

The colors shown by common fibers are: Cotton or linen, light blue; acetate or nylon, pale greenish yellow; cuprammonium, dark blue; viscose, lavender; vinyon, very pale blue; wool, yellow; raw silk, black; degummed silk, brown. The stain is also useful in the identification of films of cellulose acetate or viscose (cellophane), giving the above colors. The colors realized with any such dye mixture will depend somewhat on the history of the sample tested, and increased confidence follows a check by other dye mixtures.

Shirlastain (45)

When the fibers have been freed from size, filling, dyestuffs, etc., they are thoroughly wetted out with water and immersed in cold Shirlastain A for one minute, after which they are thoroughly washed in cold water.** Color reactions of various fibers are listed in Table XXIX, but frequent users are advised to prepare a set of authentic standards for comparison.

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* Texchrome, as well as the bleaching agent "Texstrip," is available from Eimer and Amend, 633 Greenwich St., New York, N. Y. 10014.

** Shirlastains are manufactured by Shirley Developments, Ltd., 52-56 Market St., Manchester 1, England.

TABLE XXIX

SHIRLASTAIN FIBER IDENTIFICATION

Fiber	Shirlastain A	Shirlastain B
Raw cotton	Pale purple	Greenish stone gray
Scoured cotton	Pale lilac	Dull shell pink
Mercerized cotton	Dull crocus	Copper pink
Boiled linen	Dark purplish gray	Deep Gault gray
Full bleached linen	Dull Parma violet	Gault gray
Raw hemp	Dark purplish gray	Dull moss green
Bleached hemp	–	Gault gray
Raw ramie	Pale lilac	Lichen green
Bleached ramie	–	Grayish pink
Raw jute	Mace	Olive green
Bleached jute	–	Sage green
Viscose rayon	Cyclamen pink	Maize
Cuprammonium rayon	–	Shell pink
Wool (cold)	Rust or copper	–
Wool (at boil)	Gold	–
Raw gum silk	Brown	–
Degummed silk	Dull marigold	–
Nylon (cold)	Bright golden yellow	–
Nylon (at boil)	Bright copper brown	–

Shirlastain does not differentiate completely among the various cellulose fibers, but further differentiation is possible by the use of Shirlastain B, in addition to Shirlastain A. The results, also shown in Table XXIX, indicate that with Shirlastain B all the bast fibers take up shades of green, and, with the exception of ramie, these green shades are obtained even when the fibers are fully bleached. Recently, two new types, D and E, were added to the series of Shirlastains; in addition, an acidified mixture of A and D is useful for confirmation of identifications (125).

If the size of a fabric contains gelatin and the fabric is stained with Shirlastain A before desizing, the sized threads assume the color of brown. Most viscose and acetate rayon yarns are sized with gelatin sizes, and the colors of the sized and unsized yarns are so different that staining with Shirlastain A is a fairly sensitive test for the efficiency of desizing.

DIFFERENTIATION BETWEEN ANIMAL AND VEGETABLE FIBERS

Liebermann's Method (46)

A saturated aqueous solution of fuchsin is treated dropwise with sodium or potassium hydroxide until the solution becomes colorless; after filtering,

the solution is stored in a tight stoppered bottle. The fibers are placed in this solution, heated and rinsed. Animal fibers are colored red, whereas vegetable fibers remain colorless.

Millon's Reagent

Dissolve one part of mercury by weight in one part of cold, fuming nitric acid (sp. gr. 1.42) by weight. Dilute with twice the volume of water and decant the clear solution after several hours. A few drops of the reagent are added to the fibers on the slide and allowed to stand for two minutes. Animal fibers become pink to red, whereas the vegetable fibers remain colorless.

Picric Acid Reagent

One gram of picric acid is dissolved in 100 ml. of distilled water. The fibers are placed in a few drops of this reagent on the slide and allowed to stand for three minutes, after which they are washed with water. Animal fibers are stained a brillant yellow, whereas the vegetable fibers are not dyed.

Test for Wool (47)

The detection of wool in the presence of cellulose fibers, when the characteristics of the wool fibers have been destroyed or the fibers have been dyed a dark color, is very difficult. In such cases the following method has been found satisfactory.

The fibers on the slide are covered with two drops of 30% sodium hydroxide solution and gently heated over a flame until vigorous boiling begins, whereupon the slide is removed immediately, and examined under the microscope. The wool fibers become greatly swollen and may be partly dissolved, and present the characteristic appearance of being full of cells or bubbles. Undyed wool gives a dirty yellowish-brown color. Cotton and wood pulp fibers are unchanged, except that they become somewhat clearer and somewhat shrunken.

DETERMINATION OF COTTON, LINEN, AND WOOD FIBERS (48)

The differentiation, especially between linen on the one hand and cotton and wood fibers on the other, is greatly facilitated by staining with silver nitrate solution, clearing with nitric acid, and examining under polarized light.

The disintegrated pulp sample is heated 30 minutes in a boiling water bath with ammoniacal silver nitrate (10 ml. of 5% silver nitrate solution to which has been added just enough 20% ammonium hydroxide solution to dissolve the silver hydroxide first precipitated), filtered through a small, tough filter, and washed thoroughly with distilled water; 10 ml. of alcohol

are added and the pulp is stirred gently, drained, and the alcohol treatment is repeated. The filtered pulp is dried, removed from the filter paper, and teased out thoroughly with a fine forceps and small scalpel; it is then added to 20 ml. of 2% nitric acid previously heated to 70-80°C., stirred frequently for five minutes, filtered quickly, and washed thoroughly with distilled water, diluted to the original concentration, and made into slides. Working with known mixtures of linen and cotton, the error found was from ± 1.4-2.6%. With mixtures of cotton, linen, and wood fibers the errors were somewhat larger, but were still considerably smaller than those generally reported by the estimation method. Many other fibers, such as hemp, manila, and esparto, stain like linen. The behavior of cotton, which does not become stained, is thus exceptional.

DIFFERENTIATION BETWEEN COTTON AND LINEN (49)

Chrysophenin and Safranin

The fibers are stained to a dense rose color in warm safranin solution, washed with cold water, and then placed in a cold solution of chrysophenin to which has been added a little soda. The linen or flax becomes light reddish brown and the cotton yellow with traces of red. Dyed in this manner cellulose becomes yellow, wool and silk carmine red, jute and manila scarlet red, and hemp dark reddish brown. If an additional bath of alkaline benzoazurin is used at room temperature, cellulose will be dyed blue, cotton yellow, flax reddish, and silk, wool, jute, manila, and groundwood red.

Cochineal Tincture

An alcoholic extract of cochineal stains cotton light red and flax violet.

Copper Sulfate

The fibers are placed in a 10% copper sulfate solution, thoroughly washed, and then put into a 10% yellow potassium ferrocyanide solution. Cotton remains colorless while linen stains red brown.

DIFFERENTIATION OF COTTON, FLAX, AND HEMP (50)

The dye bath is made with 0.7 g. of toluylene orange G, 0.3 g. of benzo pure blue, and 10 g. of sodium sulfate in one liter of water. The fibers are quickly drawn through the solution and blotted. When dry, the cotton is yellow; flax, olive yellow; and hemp, green.

DIFFERENTIATION OF COTTON, FLAX, HEMP, AND JUTE (51)

The fibers are treated with a neutral solution of safranin, washed with

water, and then dyed with a weak alkaline solution of chrysophenin. Cotton becomes yellow with red spots; flax, white; hemp, dark brick red; jute, red; and wool, crimson red.

If a solution of benzoazurin is now added, the yellow of the cellulose turns blue; cotton, yellow; flax, reddish. Similar results are obtained by combination coloring with benzo brown and Congo red, with Malachite green in neutral solution, and chrysophenin and benzopurpurin in a weak alkaline solution.

DIFFERENTIATION BETWEEN UNBLEACHED AND BLEACHED COTTON (46)

One-tenth gram of cotton is boiled in 10 ml. of 3% solution of Victoria blue B (based on weight of fiber). It is washed for one minute in cold water until the water is practically colorless, again boiled for one half to one minute in distilled water, and finally rinsed in cold water. Unbleached cotton will be dyed deep dark blue and bleached cotton will show very little color. Control samples should be used.

DIFFERENTIATION OF BAST FIBER STRUCTURES (49)

Bast fibers are immersed for several hours in a 10% acidified stannic chloride solution, washed thoroughly, and treated with 10% gold chloride solution; the fibers are colored brown and then brownish red. The reaction can be applied to jute, hemp, flax, and typha.

DIFFERENTIATION BETWEEN FLAX AND HEMP

The fibers on the slide are first boiled with a grain of Malachite green in acetic acid, cooled, the solution blotted off, the fibers washed twice with water, and treated with a lukewarm solution of benzopurpurin 10B. Flax fiber is stained red and the protoplasm in the lumen green; hemp is varicolored with impure mixtures of green, blue and violet; cotton, purple red; and groundwood, jute, and manila, green (11).

Cross Sections (52)

Cross sections of the fibers are placed on a slide and treated with ruthenium red (0.01% solution). After five or ten minutes, the sections are washed and examined. The intercellular substance and the cell contents are stained red. In flax, the cell contents are apt to be more prominently stained; in hemp, the intercellular substance. Cross sections of hemp fibers tend to be somewhat flattened and to have an elongated lumen with projecting points, whereas sections of flax fibers have a more nearly circular lumen and cell wall.

Cuprammonium Solution (53)

Two grams of cupric hydroxide are dissolved in 100 ml. of 25% ammonium hydroxide and the solution is kept in a dark bottle. Fibers placed in a drop of the reagent should be under the microscope in order to follow the stages of swelling immediately observed. As the flax fibers swell the protoplasmic remnants become prominent in the tortuous narrow central tube, whereas the inner canal of hemp exhibits prominent horizontal striations. This test is qualitative only because swelling occurs at different rates in individual fibers, and all fibers do not exhibit the distinguishing characteristics. If the fibers are highly bleached the test may fail. Swelling may be arrested by replacing the cuprammonium solution with a solution of equal volumes of glycerin, alcohol, and water.

Cyanine (54)

A saturated solution of cyanine is prepared at room temperature and diluted somewhat with water and then with a third of its volume of glycerin. The fibers are macerated by boiling in 1% sodium hydroxide solution and thoroughly washed. They are then heated in the cyanine reagent on the slide. After staining the fibers, it is well to wash them carefully with a solution containing one volume each of glycerin, water, and alcohol, before mounting them in glycerin. The flax fiber wall is colorless, with scattered blue spots from intercellular substance, if present, and blue, threadlike protoplasm in the lumen. In the hemp fiber wall the primary layer is green blue and the secondary layer is colorless to weak blue; the intercellular substance is blue, but not prominent because of the color of the primary wall; the protoplasm is blue and broken, but seldom threadlike.

Moisture Test (55)

A very simple test is performed by drawing a fiber between moist finger tips. When the fiber is held in a vertical position and the tip is viewed from above, the flax fiber is seen to move in a clockwise direction, whereas the hemp fiber may move either in a clockwise or a counterclockwise direction, usually the latter. The motion is much weaker in hemp than in flax, possibly because of differences in the layers of the fiber walls of the two species mentioned in Chapter VII.

Potassium Dichromate (56)

Potassium dichromate is dissolved in an excess of sulfuric acid and the solution diluted somewhat with water. The fibers are immersed in the reagent on a slide and placed at once under the microscope in order to observe the swelling. Flax swells somewhat more readily than hemp. The central canal of flax is quite wavy, whereas that of hemp is practically straight. The fiber should be observed when it is drying, not as it is moistened. If a bundle of fibers is used, an appreciable time may be required for the liquid to penetrate the fibers; therefore, the first twisting will be the result of the moistening

of the fibers, and will, of course, be in the reverse direction to that observed when the fiber is drying. Most of the cordage fibers respond to this test in the same manner as hemp. Ramie and related fibers twist clockwise on drying, as does flax.

DIFFERENTIATION OF JUTE, PHORMIUM, AND HEMP (57)

The fibers are left for one minute in chlorine water to which has been added a few drops of ammonia. Jute and phormium are initially light red and later dark brown; flax and hemp are orange or light brown.

DISTINCTION BETWEEN MITSUMATA AND GAMPI (58)

Microscopically, the Japanese fibers mitsumata and gampi are very similar, but if the sample is cut into very small pieces, disintegrated in 1% caustic, distributed on a microscope slide, and a few drops of 17.5% sodium hydroxide added, mitsumata can be differentiated from gampi by its beadlike structure.

DIFFERENTIATION BETWEEN MANILA AND SISAL

The Swett Test (59)

A bleached solution is prepared by adding one part of calcium chloride to seven parts of water and filtering. Thirty milliliters of the solution is acidulated with two milliliters of glacial acetic acid.

Most of the spinning oil is removed from the fibers by pouring ether down the strand, and the ether is evaporated by waving the strand in the air for a minute or two; one end of the sample is immersed in the acidulated bleach solution for 20 seconds, rinsed with water and then with alcohol, and finally immersed in concentrated ammonium hydroxide. Manila will turn brown instantly and other hard rope fibers will turn cherry red.

When applied in this manner, the test is somewhat fugitive, the red color degrading in a few minutes. When it becomes necessary to estimate the percentage of manila fibers, the test is applied as before but, instead of immersing the fibers in ammonia, the treated ends are suspended above the ammonia for a minute or two. In this way the manila does not assume the brown color as rapidly, but at the end of two or three minutes the color develops and is permanent.

The Amoa Test (60)

Take a piece of the sample to be tested, whether in the form of twine, yarn, or rope, immerse it in a boiling 5% nitric acid solution and allow it to steep 5-10 minutes. Remove and rinse, then place in a cold solution of

one volume of 52°Tw. sodium hypochlorite in three volumes of water and keep the sample in this solution about ten minutes to develop the colors. Remove the sample, separate the fibers, and dry. Manila becomes a bright orange-red; sisal and other hard rope fibers become a pale yellow color. Also, after drying, the manila fibers have a high gloss, while the other fibers are left with a dull lifeless appearance.

Dave and Mehta (126) used a similar method on a one-gram rope sample: Extract 8 times with ethyl alcohol, boil 2 minutes with 5% nitric acid solution, and wash out acid with distilled water; treat with 100 ml. calcium hypochlorite (3.5 g./l. available chlorine) for 10 minutes at room temperature. Manila is dyed deep orange and sisal lemon yellow.

MICROSCOPIC EXAMINATION OF DAMAGED COTTON AND WOOL FIBERS

DAMAGED COTTON HAIRS (61)

Congo Red Test

About 0.1 g. of cotton is placed in a water bath in a filter flask and the air withdrawn by a filter pump. The cotton is then gently squeezed to remove most of the water, placed in 25 ml. of 11% sodium hydroxide solution, shaken thoroughly, and allowed to stand for five minutes. After washing rapidly with water, it is placed in 2% Congo red solution and shaken at intervals for six minutes. It is removed and washed by shaking with water, the latter being changed until it no longer becomes pink. The cotton is placed as quickly as possible in 18% sodium hydroxide solution, teased out, and a few hairs mounted in the same liquid for microscopic examination. To prevent any liquid from escaping, the cover glass is sealed. The behavior of the cotton is tabulated in Table XXX.

TABLE XXX

APPEARANCE PRODUCED BY THE CONGO RED TEST OF COTTON HAIR IN DIFFERENT STAGES OF DEGRADATION

Degree of Damage	Attacked by Fungus	Exposed to Heat	Mechanical Damage	Treated With Sulfuric Acid
None	Stained pink	Stained pink	Stained pink	Stained pink
Slight	Narrow multiple red spiral bands	Broad simple red spiral bands	Surface bruises	-----
Moderate	Stained red evenly	Narrow multiple red spiral bands	Deep cuts	Irregular red patches
Severe	Stained red and cracked	Stained red and cuticle singed	-----	-----

NORMAL AND MILDEWED COTTON FIBERS (62)

About 0.1 g. of cotton fiber is treated with about 15 ml. of 0.15% Victoria Blue B solution to provide an excess over that required for complete wetting of the sample; this operation is conveniently performed in an 8-inch pyrex test tube. After boiling for one minute, the sample is washed with cold water until no color is removed and is then boiled with freshly distilled water until no further bleaching of the color is observed; it is then rinsed with cold water and dried on filter paper.

After experimentation to determine the concentration which will cause swelling without rapid solution, a few fibers are treated with cuprammonium solution during microscopical observation to study the rate and manner of swelling.

Normal samples of sound raw cotton will usually be stained an even dark purplish blue. In the case of mildewed specimens, however, the stained samples are usually mottled with a lighter or clearer blue, probably because the dye acts as an indicator, being blue to the acid range and purple to the alkaline range. As the mildewed spots usually are acid, they appear light blue in the stained sample. Microscopically, the swollen fibers present a morphological picture similar to that obtained in the swelling test of Thaysen and Bunker (63). The normal fibers have a beaded appearance. In the mildewed fibers, swelling takes place more readily and the whole fiber swells evenly.

DETECTION OF DAMAGED WOOL (64)

Chlorine Water (65)

Scales of wool are not easily attacked by chlorine water, whereas fiber cells and elasticum react readily. Swelling caused by the reaction of the latter forms globular enlargements on the fibers, the scales being distended. Place the fibers on a slide, cover with a few drops of water, add an equal volume of chlorine water, and examine at 200X magnification.

Indigo Carmine (54)

The fibers are placed in a saturated solution of indigo carmine acidified with sulfuric acid, left for a short time, and then mounted in glycerin. The damaged portion of the wool stains bright blue but the undamaged portions are unaffected. If this treatment is followed by a picric acid solution, the undamaged parts assume a yellow color and the damaged parts turn green.

Potassium Hydroxide (46)

Dissolve 20 g. of stick potassium hydroxide in 50 ml. of concentrated ammonia with shaking and while cooling with running water. Allow to stand

to permit the escape of excess ammonia, which is annoying in microscopic work. The solution may be kept indefinitely. Acid-damaged wool swells, at first very strongly, partially curls, and after a short time, according to the degree of damage, rollbacks of the epidermis occur in the slittings.

Standardized Method (66)

About 0.1 g. of wool is purified by extraction in alcohol and ether, and immersed in 100 ml. of sodium hypochlorite solution (0.12% chlorine; pH 10) for 15 minutes at 20°C., with agitation. It is then removed, washed for one minute in each of five changes of water (200 ml. each), transferred at once to a bath of 100 ml. of 0.04% methylene blue solution for five minutes, after which it is washed in running water for five minutes, and dried between filter papers.

The stained fibers are cut into lengths of about 1 mm., about 1000 fragments placed on a microscope slide, covered with liquid paraffin, and examined at 200X magnification. A stop is placed in the eyepiece to limit the field of view to a band about 50 μm. wide, and only fibers examined across the band and making an angle of more than 45° with the length of the band are to be counted. The following classification is adopted:

Class	Appearance of Fibers
0	No sign of attack.
1	Scale edges only attacked; stained area extending down the scale for not more than one quarter of the maximum scale length.
2	Any signs of damage other than those shown in Class 1, but not extending over the whole surface, e.g., blotchiness.
3	Complete staining of scales, but scale edges still well marked.
4	Heavy general damage; scales not clearly visible.

STAINS FOR SIZING AND COATING MATERIALS IN PAPER

ROSIN SIZING

Raspail Test

Method 1. This test depends on the fact that protein, rosin, fats, etc., are colored rose or violet by the action of concentrated sulfuric acid

and a strong cane sugar sirup. The procedure is to dampen a small piece of the paper with the sugar solution, place it on a slide, free it from excess of sugar solution by lightly pressing with blotting paper, and then add sulfuric acid when the paper is under low-power magnification. No visible reaction occurs with animal sizing but, if rosin sizing is present, a deep red color will appear in those portions of the paper in contact with acid.

Method 2. Add equal volumes of glacial acetic acid and strong sugar solution. Spread a drop of this mixture on the paper and allow it to dry. Then add a drop of concentrated sulfuric acid. Depth of color is in proportion to the amount of resin present (67).

Method 3. Papers which have been starch sized may be treated with a mixture of three parts concentrated sulfuric acid, two parts glycerin, and one water, followed by a concentrated sugar solution and then by concentrated sulfuric acid (67).

Potassium Permanganate (68)

Rosin size in paper may be recognized by disintegrating the paper with pure water and adding a little potassium permanganate solution. A brown color develops due to the action of the permanganate on the resinate. When the fibers are boiled with pure water and stained with zinc chloride-iodide, the rosin appears as yellow spots when viewed under the microscope. If the paper is disintegrated with potassium hydroxide solution the rosin is not colored. The reaction fails in the presence of mechanical wood pulp.

GLUE SIZING

Tannin Test

A piece of paper is wetted, placed on a slide, warmed, and the paper removed. If tannin solution is added to the aqueous extract remaining on the slide, no precipitate appears if the sizing material is rosin, but a voluminous precipitate, readily observed under the microscope, is formed in the case of animal size.

Biuret Test

Albuminous bodies, treated first with potassium hydroxide solution and then with copper sulfate solution, are colored red and violet. A small piece of paper is wetted on a slide with 10% potassium hydroxide solution and, after a minute or two, during which the alkali dissolves the size, one drop of 2% copper sulfate solution is added from a glass rod. The violet shows best in the fiber under the microscope after the slide has been drained by tilting on its edge. Rosin size gives a fully negative result.

Graff's Method

Strips of paper, at least two by four inches, are put in an enamel tray containing 2.5% copper sulfate solution and left for ten minutes at room temperature. The paper is removed and pressed between clean blotters, then transferred to another tray containing 2% sodium hydroxide in 50% alcohol, and left for two minutes. The papers are removed, blotted, and dried. Not only is the intensity in color proportional to the amount of glue in the paper, but the stain shows if the glue has been added in the beater (complete penetration), or in the tub (surface). The reaction also shows whether the glue size has been hardened by dipping in a formaldehyde bath or whether the formaldehyde has been applied to one side of the sheet only; the uniformity of penetration of the glue is also indicated.

Iodine Test (69)

If paper is teased apart with a needle on a microscope slide and dabbed with iodine solution (1% potassium iodide solution saturated with iodine), a brown color is produced when animal or rosin size is present. If, however, the paper is heated with a little water on the slide and then removed, the extract will show a characteristic appearance under the microscope, which varies with the nature of the sizing agent. Animal-sized papers leave a considerable residue, which is gradually dissolved by the iodine, producing a rusty red color. Rosin-sized papers leave a light grainy residue with an uneven dented edge around the space on the slide formerly covered by the water solution. This residue forms a brown color with the iodine solution.

CASEIN SIZING

Adamkiewicz Test

If albuminous bodies are warmed with a mixture of two parts of glacial acetic acid and one part of concentrated sulfuric acid, many of them give a strong red or violet color. Casein shows this reaction but animal size does not. The paper is wetted on the slide with dilute potassium hydroxide solution and then removed with a needle. The extract is acidified and warmed to coagulate the casein, which is washed by alternate moistening with water and pressing with filter paper, and then treated with the mixed acids.

Millon's Reagent

This reagent shows red coloration with casein but none with glue. It is prepared according to directions on p. 270, or by dissolving 20 g. mercuric nitrate in 20 g. concentrated nitric acid in 160 ml. distilled water. In the second method 1-2 ml. of 1% sodium or potassium nitrite solution must be added.

GELATIN SIZING

In some papers waterproofed by the gelatin-formaldehyde process, the gelatin can be detected directly by means of an acid dye (Lanafuchsin), which gives a violet red color with gelatin but leaves the fibers colorless.

According to Preston (70), gelatin may be detected very easily by staining in the following solution: Acid green, 2 g.; mercuric chloride, 5 g.; glacial acetic acid, 5 g.; distilled water, 88 ml.

This solution does not keep, apparently due to interaction of the dye with the mercuric chloride. It is better to use two solutions and mix equal volumes immediately before use. One solution is made with two grams of acid green dye in 98 ml. of distilled water; the second solution contains 5 g. of mercuric chloride, 5 g. of glacial acetic acid, and 90 ml. of distilled water.

The paper to be tested is allowed to remain in the cold stain solution for five minutes, removed, washed thoroughly with running water for about 15 minutes, and then examined. Gelatin stains an intense green, while cellulose remains colorless. Acid violet R may be substituted for acid green if, for any reason, a violet stain reaction is preferred.

LATEX SIZING

About 200 $cm.^2$ of the paper to be tested is torn into fairly small fragments, put into a small wide-mouthed flask, and covered with about 50 ml. of chloroform. It is allowed to stand for an hour with occasional shaking, and then gently warmed for 15 minutes (preferably on a water bath), again with occasional shaking. The paper is removed and the chloroform evaporated until only 2 or 3 ml. remains; this is now a solution of rubber and rosin, if these materials were present in the paper. Other substances will not be affected. The solution is poured into a small test tube and warm glacial acetic acid added until a white cloud is produced, which persists on shaking. On standing, a coagulated precipitate of rubber forms, if rubber is present in the paper. The rosin remains in solution. Viewed microscopically, the precipitate consists of globules, which flatten when the cover glass is pressed, and which readily coalesce. The presence of a permanent precipitate, or cloud, if the amount is very small, is sufficient, however, to prove the existence of latex in the paper. The test can be made quantitative with a known weight of paper (71).

STARCH SIZING

Starch Retention

The stock solution for this test contains 0.9 g. of potassium iodide and 0.65 g. of iodine in 50 ml. of distilled water. For use it is diluted with ten parts of water. One-inch strips of paper are dipped in the diluted solution for 15 seconds, rinsed with distilled water for 5 minutes, and dried. The resulting color will vary in density according to the relative amounts of starch retained by the paper. The color will show slight variations depending

upon the type of starch used for sizing and coating. Differences in the retention by wire and felt sides of tub-sized paper can be detected.

When the starch retention is over 1%, it is possible to distinguish between tub- and beater-sized papers; the former are stained a much deeper shade, which varies from a light to a dark reddish blue, whereas the latter have a pale bluish-white color.

The density of the color reactions of the stained sheets makes it possible to determine quickly the percentage of starch retained by the paper, in steps of 1%, by comparing the samples with a standard color chart. This applies to both tub- and beater-sized papers. The hue of the color makes it possible to determine the kind of starch used, e.g., corn, potato, tapioca, etc.

Microscopic examination of stained samples shows the relative distribution of starch on and between the fibers. In tub-sized papers one can observe the uneven distribution as to wire and felt sides of the paper and the spotted distribution sometimes produced by high concentration of starch. Some idea of retention in beater-sized sheets can also be obtained when boiled starches are used.

Starch and Dextrin Differentiation (67, 72, 73, 127)

Starch grains from various plants have characteristic forms and sizes by which they can be recognized. However, during the process in which they are converted into "size," the grains burst and lose their characteristic appearances. Similarly, dextrin, which in the dry state may or may not preserve the original appearance of the starch grains from which it was made, in "size" lacks any possibility of morphological characterization. Consequently, the only possible microscopical method for identification of starch size is by means of a staining reaction.

A very dilute solution of iodine in dilute potassium iodide solution is a convenient stain; it gives a blue color with starch and a purple to brown color with dextrin. The exact color with the latter substance depends on the degree of depolymerization; the more it is depolymerized the less blue is the color.

RESINS AND PLASTICS

Since the close of the Second World War the use of synthetic resins and plastics in the paper industry has expanded tremendously. Although the identification of these materials frequently involves considerable chemical testing it is believed desirable to mention that microscopical examination may be helpful in combination with certain spot tests or special techniques, such as fluorescence microscopy (128, 129).

Wet-Strength Resins

Urea-formaldehyde and melamine-formaldehyde resins are by far the most important ones used to impart wet strength. It is natural then that major efforts for identification have been directed toward these substances.

In 1945, Stafford and co-workers (74) described a dye staining technique for the identification of melamine wet-strength resin samples in the presence of samples containing urea-formaldehyde resins as well as other wet-strength agents. This method was revised by House and Woodberry (75).

This test is based upon the fact that acid dyestuffs are not substantive to cellulose, and unless mordanted, can easily be washed from cellulose fibers. On the other hand, the acid dyes combine with "proteinlike" substances such as wool, glue, casein, and amino-aldehyde resins. One of these acid dyes, Calcocid Alizarine Blue S.A.P.G., has been chosen as being particularly useful. Under specified conditions, untreated paper can be quickly distinguished from paper containing materials such as proteins, urea resins, or melamine resins. By using three different modifications of the same technique, applied to separate portions of the unknown sample, it is generally possible to decide whether or not melamine is present or to obtain indications as to the nature of the other nitrogen-containing substances if melamine is not present.

Widmer (76) has discussed several possibilities for the identification of melamine and urea resins in wet-strength paper. The xanthydrol test for urea has been modified to prevent interference of rosin. The paper is refluxed with 10% acetic acid, filtered, and the filtrate treated with 0.25 ml. of a 1% solution of xanthydrol in methanol. The solution is evaporated to dryness on the steam bath, the residue transferred to a small test tube, dissolved in 0.25 ml. of pyridine, and cooled slowly. In the presence of urea, a white crystalline precipitate is formed, which shows crystals of characteristic forms upon microscopic examination.

Jayme and Branscheid (77-79) have described a simple method for the detection of the presence of urea or melamine in paper. A rather concentrated solution of sulfuric acid in which phenylhydrazine is dissolved (either 1% phenylhydrazine in 40% acid or 2% phenylhydrazine in 30% acid) is transferred by means of a glass rod to the paper to be tested. After 30 seconds a drop of 10% ferric chloride solution is added with another glass rod. After a few minutes the spot assumes a deep red color if urea, and a light red color if melamine is present. Melamine requires a longer reaction period and gives a lighter shade of red, so that the two resins can be easily differentiated from each other. When the paper has not received any wet-strength treatment, a yellow color appears. The test is based on formaldehyde so casein and glue-sized papers containing formaldehyde give positive tests.

In a series of experiments, Graff (80) has shown that the sizing and resin materials used in a white paper can be satisfactorily determined by first applying the Raspail test, then the iodine test, then any one of the selective dyeing reagents (Neocarmin, Shirlastain, Colotex B, and Texchrome), and then finally checked by determining by which method the paper most easily disintegrates (0.5% caustic, 5% alum and 0.5% caustic, or acetone and 0.5% caustic).

Applying these methods to the papers tested, the results showed that the papers have been qualitatively separated into the following distinct groups: Waterleaf sheet; polyvinyl copolymer; polyvinyl chloride-acetate and cellulose acetate butyrals; chlorinated rubber; polyvinyl acetate; polyvinyl butyrals; ethyl cellulose; starch and alum; phenol-formaldehyde; urea-formaldehyde; rosin, alum, starch, and glue; melamine; and rosin, alum, and starch.

Polyvinyl Alcohol (81)

Polyvinyl alcohol gives a deep blue color with iodine-potassium iodide solution just as starch does. It will not, however, give a positive test with Fehling solution as starch does.

To make other tests for polyvinyl alcohol, scrape off the coating from a generous sample of paper by means of a safety razor blade and boil in distilled water for at least ten minutes. A portion of the water extract is evaporated in a watch glass to dryness over a water bath when a thin film will be formed. To this film a small drop of water is added together with a small quantity of powdered borax, these are mixed together with the end of a platinum wire and if polyvinyl alcohol is present the whole will form into an unmistakable semiopaque gel.

If a 2% gallotannic acid solution is added in excess to the cooled water extract a brown precipitate of colloidal nature is formed.

DYESTUFFS

An informative article by Laughlin (82) will serve as a guide to those in the paper industry who have need of a quick, simple and fairly reliable method for determining the dyestuff or type of dyestuff with which a piece of paper has been beater dyed.

STAINS FOR FILLERS AND PIGMENTS

TAPPI Suggested Method (83, 84)

Procedures are given for the recognition of diatomaceous earth, talc, calcium sulfate, calcium carbonate, and compounds containing the ions of calcium, zinc, barium, and titanium. Other less common mineral fillers may sometimes be detected by variations of these procedures or by other chemical and optical tests found in the literature. Particular care should be used in examining fillers containing titanium dioxide, which because of its high index of refraction and small particle size can sometimes mask small quantities of other fillers, such as clay, so that the latter are difficult to identify separately. The test specimen shall be a representative sample of the paper ash such as obtained in TAPPI Standard T 413 m.

Beckh's Method (85)

A drop of the dye combination is added to the ash on a microscope slide, both are well mixed with a needle, and the mixture spread out. The loading material precipitates the dye most nearly related to it, and the supernatant liquid is drawn off with a piece of blotting paper.

The use of methylene blue as basic dye and azo acid red as acid dye will result in a distinct blue color if kaolin, talc, or asbestos are present and a distinct red if barium sulfate or plaster of Paris are present.

If the ash is colored blue by the dye, place a drop of cobalt nitrate solution on a small piece of the paper under investigation and hold it in a Bunsen burner flame until the paper is completely white; if a blue spot (Thernard's blue) is formed, kaolin may be assumed. Asbestos and talc are distinguished under the microscope as the former has long slatelike or flaky fragments of fibrous stratum.

If the ash is colored red, plaster of Paris can be readily recognized by the characteristic crystals of gypsum which form, particularly at the edge of the spot. Blanc fixe dissolves when heated in sulfuric acid, and on cooling beautiful crystals separate. Carbonates are best determined by adding acid to observe the escape of carbon dioxide as well as by studying crystal shapes.

Kollmann's Method (86)

A few dyes applied directly to paper colored only the secondary constituents (loading materials, free rosin, resinates, alumina, starch) distinctly and rendered them clearly distinguishable from the undyed fibers. Further differentiation of loading materials on one hand and sizing substances on the other was obtained by two samples of the same paper being dyed and compared. The sizing substances and aluminates were removed from one sample by alcohol, ether, and hydrochloric acid. An "ordinary" (untreated), and "acidified" (extracted with hydrochloric acid), and "ether extracted" (extracted four times alternately with alcohol and ether), and a "completely desized" (treated with hydrochloric acid, alcohol, and ether) paper were thus compared. Capri blue and Ergonon violet were the only dyes tested which proved practicable (Table XXXI).

If the paper is first dyed with Ergonon violet and then with Capri blue, the violet-colored alumina compounds can be distinguished from the kaolin particles dyed blue. The majority of the fibers remain almost colorless when the Capri blue solution has the correct concentration, does not stay in contact too long, and is washed sufficiently after dyeing. It is thus possible to ascertain the local arrangement of the amorphous elements of paper which are otherwise invisible.

TABLE XXXI

COLORS PRODUCED BY CAPRI BLUE AND ERGONON VIOLET

Dye	Ordinary Paper	Ether Extracted	Acidified	Completely Desized
Capri blue	Fibers undyed except individually once, many intensely blue amorphous elements	Same	Same	Same
Ergonon violet	Many very pale violet, rarely intensely violet dyed amorphous elements	Almost all covered by intensely violet amorphous elements	Almost unnoticeable rose color, many amorphous elements	Individual pale orange-yellow amorphous elements

STAINS AND REAGENTS FOR SPECIAL PURPOSES

MICROSCOPE EXAMINATION OF PULPS FOR RESIN

Sudan IV

The stain is prepared by saturating a mixture of three parts of alcohol and one part of water with the dye and adding two parts of this solution to one part of glycerin. The method of staining with Sudan IV, as well as with the dyes mentioned later, depends upon the degree of delignification of the pulp. For unbleached pulps, three or four drops of the stain are spread over the fiber field and allowed to stand for at least an hour, after which the cover glass is placed over the fibers, and the surplus stain is drained off. For bleached pulps the stain is allowed to react for only two or three minutes before the cover glass is put in place. Resins and fats are stained red, whereas the fibers remain colorless.

Potts (87) has suggested the following method of using Sudan III, the predecessor of Sudan IV. The pulp is stained with 0.2% solution in 70% alcohol for one hour, washed rapidly with 50% alcohol, cleared with 50% glycerin for 15 minutes, and mounted in glycerin jelly.

Several dyes superior to Sudan III have been found for the identification of pitch (88). Sudan Orange 2R stains pitch red and can be used with daylight illumination; Sudan Black stains pitch black and indophenol gives a blue color reaction. These dyes are very suitable for examination of specimens by artificial light.

The solutions are prepared as follows: Sudan Orange 2R and Sudan Black are dissolved in 72% alcohol-glycerin mixture (3 volumes of alcohol and 1 volume of glycerin) to form a saturated solution. Indophenol is dissolved in the alcohol-glycerin mixture until a saturated solution is formed, and one volume of this solution is mixed with one volume of 72% alcohol and one volume of glycerin. Indophenol is very sensitive to acids and therefore samples used with this dye must be free from acid.

Noll has developed another method for dissolving these three dyes. One-tenth gram of the dye in 50 ml. of a solution composed of 20 ml. of alcohol, 20 ml. of glycerin, and 10 ml. of water is heated slowly with constant stirring until it simmers but is not permitted to come to a boil. The solution is cooled to room temperature and carefully filtered; no consideration is given to the fact that some undissolved dye remains on the filter. The filtered solution is kept in glass-stoppered bottles. If, on standing or cooling, the solution should deposit a precipitate of the dye, it must be refiltered.

It has been proposed recently to use isopropyl alcohol instead of ethyl alcohol with various Sudan dyestuffs; 0.5 g. dyestuff is dissolved in a mixture of 32.5 ml. isopropyl alcohol and 17.5 ml. distilled water (130).

Orange R

The sample is boiled a few minutes in distilled water, immersed for one minute in 0.1N iodine, washed, immersed in 6N hydrochloric acid, and washed; the treatment with iodine and hydrochloric acid is repeated. The sample is placed on a slide and examined under the microscope with transmitted light. Fats and resins show as yellow concretions with the shape of wood rays. On dyeing the sample with an alcoholic solution of Orange R, fats and resins appear yellow on a white background (89).

Alkannin

A solution of alkannin (the dark red dye from alkanet root, procurable in dough form) in absolute alcohol with the addition of an equal volume of water stains fats, volatile oils, pitch, and rubber, a deep red, whereas other materials stain much less or not at all (46).

MYCELIUM REAGENTS

According to Cartwright (90) sections are slightly overstained in 1% aqueous safranin, washed with water, then covered with picroaniline blue (25 ml. saturated aqueous aniline blue plus 100 ml. saturated aqueous picric acid). The slide is warmed over a flame to simmering, washed in water to remove all visible blue, passed through the alcohols, cleared in clove oil, and mounted in Canada balsam. Lignified cell walls are red and the fungus mycelium is clear blue.

According to Galloway (91) the fibers are first warmed for one minute with lactophenol and then treated for a number of minutes in an aqueous solution of cotton blue. After washing with water the fibers are again treated with warm lactophenol to remove as much dye as possible. After mounting in lactophenol the fungi will show up blue and the cellulose will be uncolored.

According to Koscielny (92) the sample is placed in 1% safranin solution in 95% or absolute alcohol for 4-6 hours (sometimes 24 hours), washed twice with 95% alcohol, transferred to 1% alcoholic solution of aniline blue for 1-4 minutes and then put directly into xylene and mounted in Canada balsam. The fibers are stained red, and the liquified parts and mycelia are stained blue.

DIFFERENTIAL STAIN FOR LIGNIN AND CELLULOSE

Ethanolamine-Silver Nitrate Reagent (93)

Microchemical studies were made to determine the location of the potential reducing substances in the cell wall. Thin (15-20 μm.) transverse sections of air-dried wood were treated with a modification of Tollen's or silver nitrate oxidation of reducing substances in the following steps:

1. Cut sections and store in 15% alcohol.
2. Immerse in an excess of saturated chlorine water for 2 minutes.
3. Immerse in 3% alcoholic ethanolamine for 2 minutes.
4. Wash with 50% alcohol for 2 minutes.
5. Immerse in 5% aqueous silver nitrate for 2 minutes.
6. Repeat procedures 3 and 5 three times more, ending finally with a 2-minute treatment with 3% ethanolamine.
7. Immerse in glycerin.
8. Mount in glycerin jelly.

In the softwoods this staining technique produces a very deep brown to black coloration in the region of the middle lamella and an amber color in the secondary walls. Ray tissue and epithelial cells were stained very deeply like the middle lamella. The hardwoods show only a faint yellow coloration in the secondary walls, whereas the middle lamella is as dark as that in the softwoods; ray tissue and other parenchymatous cells stain heavily.

Hydriodic Acid (94)

Lignin is stained bright red and cellulose violet by treating sections of plant tissue for six minutes with an aqueous solution of sodium hypochlorite and ten minutes in a 2% phloroglucinol solution in alcohol. The sections are transferred to a watch crystal, and treated with two drops of iodine solution (0.5 g. iodine and 1.0 g. potassium iodide in 20 ml. of water) and 11 drops of a freshly prepared hydriodic acid solution for one minute. After removing the excess reagents, one drop of glycerin that has been acidified with hydriodic acid is added.

Benzidine Hydrochloride (95)

The reagent is prepared by dissolving 0.2 g. benzidine in 19 ml. of distilled water and adding 1 ml. of 25% hydrochloric acid; store in an amber

bottle. Sections containing much tannin should be washed with alcohol containing 1% hydrochloric acid before staining.

After staining the lignin with benzidine, starch may be identified in the same specimen by means of iodine in potassium iodide solution and the excess iodine allowed to evaporate. This treatment also intensifies the orange stain of the lignin. A drop of glycerin, with or without phenol, may be used as a preservative, or the stained section may be washed in alcohol and xylene and mounted in Canada balsam. This reagent for lignin is more stable than aniline salts, much cheaper than phloroglucinol, and the smaller amount of hydrochloric acid required is less apt to corrode the microscope.

OXYCELLULOSE REAGENTS

Methylene Blue (96)

The fibers are dyed either for 20 minutes in cold or for a few minutes in hot 0.1% methylene blue solution and then rinsed with hot water until bleeding stops. Pure cellulose loses the dyestuff very quickly during the washing; oxycellulose and hydrocellulose retain it stubbornly. The deeper the dyeing of the fiber after thorough rinsing, the higher is the content of oxycellulose. Lignin and pectic substances, if present, react in the same manner and can be mistaken for oxycellulose. Special caution is always advisable when estimating the methylene blue test.

Diamine Pure Blue (46)

A solution of diamine pure blue gives a strong color with pure cellulose but oxycellulose remains colorless. The sample is placed in a 0.05 to 0.1% cold solution of diamine pure blue for 20 minutes and then washed thoroughly with water; pure cellulose is very strongly colored.

Silver Thiosulfate Stain (97)

The stain is prepared by slowly adding 25 ml. of a 16% solution of sodium thiosulfate to 25 ml. of a 4% solution of silver nitrate, followed by 25 ml. of 16% sodium hydroxide solution; the mixture is brought to a boil, filtered, and water is added to a total volume of 100 ml.

The filtered solution is heated on a steam bath to 80°C., 10 g. of wet pulp (squeezed dry between the fingers) are added, the mixture stirred and allowed to remain on the steam bath for five minutes. The pulp is filtered, squeezed dry, and rinsed in 200 ml. of 0.1% acetic acid and then in a 500-ml. Erlenmeyer flask filled with distilled water; finally, sheets are formed and dried in an oven at 70°C.

Silver Hydroxide Stain (97)

Unfixed. For each ten grams of wet pulp (squeezed dry between the fingers) 75 ml. of a 10% solution of silver nitrate are precipitated with concentrated ammonium hydroxide, an excess of the latter being added until the solution becomes clear; the solution is made up to 100 ml. with water. This solution is heated on a steam bath to 50°C., the pulp added, the mixture stirred, and allowed to stand for five minutes, the pulp rinsed in 0.1% acetic acid, and then in a 500-ml. Erlenmeyer flask filled with distilled water; sheets are made and dried in an oven at 70°C.

Fixed. When the pulp has been stained, acidified, and rinsed as described for the unfixed stain, it is treated with 200 ml. of an 18% sodium thiosulfate solution at room temperature for three minutes and then rinsed; sheets are made up and dried as before.

Correlation With Other Pulp Factors

If strips of the stained sheets are arranged, as far as possible, according to the relative strengths of the stain reactions, and mounted on cards with the darkest color at the top of the card for each stain, the colors can be given arbitrary numerical values starting with one for the darkest and increasing as the relative strengths of the colors decrease. It should be pointed out that there is some difficulty in properly arranging these colors according to their relative density. This is particularly the case with the unfixed silver hydroxide stain, for which the colors, according to their relative density, form four distinct groups: Dark brown, dark grayish purple, gray, and light grayish purple.

The differences between the colors produced by the unfixed and fixed silver hydroxide stains suggests the following – the pulps contain a certain amount of sulfur or sulfur compounds, which react with the silver hydroxide to form silver sulfide; this ranges in color from brown to gray. Oxycellulose and other reducing impurities with silver hydroxide form silver oxide ranging in color from grayish brown to light gray.

The unfixed hydroxide stain does not differentiate between oxidation products and sulfur compounds when they are present together, but fixation of this stain with sodium thiosulfate removes the silver sulfide but does not affect the silver oxide; therefore, the fixed silver oxide stain must depend upon the silver oxide which is formed, and varies according to the degree of oxidation of the pulps.

If a sulfite pulp is first treated with the silver hydroxide stain and its numerical evaluation noted, and then fixed with sodium thiosulfate, the stain remaining on the fiber will be a measure of the oxidation of the pulp; the difference in numerical values of the two stains will be a measure of the sulfur compounds in the pulp.

Although these staining methods have been studied only in a preliminary way, correlations have been found between the stain reactions and the cooking stain, purity factor stain, copper number, and percentage of alpha-cellulose.

When the stain reactions are given arbitrary evaluations, the silver thiosulfate reaction is negatively correlated with the copper number and positively correlated with the percentage of alpha-cellulose. The unfixed and fixed silver hydroxide stains are both positively correlated with the cooking stain, bleach stain, and purity factor.

MERCERIZATION REAGENTS

Sulfuric Acid-Formaldehyde Test (98)

The solution is prepared by mixing 320 ml. of 69% sulfuric acid with 260 ml. of 40% formaldehyde. The sample to be tested and a known sample of mercerized and unmercerized cotton are immersed in the reagent for two minutes at room temperature. The samples are neutralized with hot dilute sodium carbonate solution and thoroughly washed. The samples are then dyed together in a very dilute boiling bath of Chlorazol Sky Blue GW made alkaline with sodium carbonate. By comparing the color of the sample to be tested with that of the other sample, mercerization can be detected.

Cuprammonium Solution (53)

The solution is prepared by dissolving two grams of cupric hydroxide in 100 ml. of 25% ammonium hydroxide, and adding a few drops of ruthenium red; store in an amber bottle. The fibers are mounted in a few drops of solution and the process of swelling is observed under the microscope. Ordinary cotton exhibits marked ballooning, the constrictions occurring between the balloons where the resistant cuticle is shoved together. The balloons appear light blue, the cuticular remnants deep pink. On the other hand, mercerized cotton exhibits no such ballooning, because the cuticle has been attacked by the mercerization process. There is an almost uniform swelling along the whole length of the fiber. The individual fibers vary in their rate of swelling, as is generally the case in such reagents, so that a quantitative differentiation would prove impractical.

Hübner's Reagent (99)

The reagent consists of 20 g. of iodine in 100 ml. of a saturated solution of potassium iodide in water. The fibers are immersed in the solution for a few seconds, and then washed several times. Unmercerized cotton is stained only slightly, whereas mercerized cotton becomes black or deep indigo.

Benzopurpurin 4B (100)

Dye the sample in a weak solution of benzopurpurin and add strong hydrochloric acid, drop by drop, to the diluted dye bath in which samples of mercerized and unmercerized cotton are lying, until the shade of the unmercerized piece just becomes blue and the mercerized sample remains a bright red color. The difference is not due to alkali remaining in the mercerized material,

for this may be treated with acid until the shade becomes blue, and upon being returned to the dye bath the red shade will reappear. A piece of cotton spotted with bleaching powder, or other agents capable of producing oxycellulose, then rinsed in acid, and finally in water, may be dyed a deep shade with benzopurpurin; it may be placed in acid so that the shade becomes blue, and then rinsed in water until the red color on the ordinary cotton reappears. Those parts which have been converted into oxycellulose will remain blue-black in color.

Methylene Blue-Potassium Iodide-Iodine (101)

The fibers are stained for a few minutes in a mixture of 0.001% methylene blue and 0.1% sodium carbonate. The fibers are then rinsed and covered in a test tube with 10 ml. of 1% (for cotton) or 3% (for linen) solution of sodium carbonate. A few drops of a solution of one gram of iodine in 100 ml. of a 20% solution of potassium iodide are then added. This solution is heated to boiling, poured off, and immediately replaced by fresh sodium carbonate solution. Mercerized material turns blue to reddish purple, the color being more or less red, according to the degree of mercerization. The redder the shade, the more complete is mercerization.

SPECIAL TESTS

Bleaching Reagents

It is sometimes advisable to bleach specimens before mounting, and sometimes also for the identification of certain wood residues or for the removal of natural or artificial coloring matters with which the fiber may have been stained.

Eau de Javelle and Eau de Labarraque (46). This reagent is prepared by mixing 20 parts of calcium chloride with 100 parts of water in one flask (shake often and let stand for a few days). In another flask mix 25 parts of potassium or sodium carbonate to 25 parts of water. When the second mixture has dissolved, both solutions are mixed together in a well-stoppered flask, allowed to stand for some days, and then carefully decanted. The reagent must be protected from light. Sections are decolorized in a few minutes; tannic acid colored objects (bark and seed particles) are quickly and completely bleached. Completely bleached objects can, after washing, again be dyed with safranin or other dyes.

Sodium Hypochlorite (101). A convenient formula for a bleach solution follows: Solution A – Calcium hypochlorite, 5 g., in 15 ml. if distilled water; Solution B – Sodium carbonate, 5 g., in 15 ml. of distilled water. Decant the clear liquor from A and add this to B, allow to stand for one hour to settle and then filter the solution for use.

Hydrogen Peroxide (101). For use the solution is made up as follows: Hydrogen peroxide (10 vol. strength), 5 ml.; distilled water, 100 ml.; and ammonia, 0.5 ml.

Differentiation Between Genuine and Imitation Parchment

The paper is cut into strips about half an inch wide and immersed in hot water for some time. Parchment paper does not swell and its strength is not impaired. The tear of the treated sample is as smooth as if it had been cut, and under slight magnification the edge will be seen to be slightly ragged. Imitation parchment, on the other hand, swells in water and, after a short period of immersion, loses practically all its strength. On tearing, the fibers can be distinguished with the naked eye; and when the tear is examined under low power the fibers are quite apparent, as in the case of ordinary paper.

On immersing white parchment paper in lime water its color remains unchanged, whereas imitation parchment turns brownish yellow and the color persists even after washing.

A solution of 0.1 g. of iodine and 0.5 g. of potassium iodide in a minimum amount of water, added to 10 ml. of zinc chloride solution (sp. gr. 1.526), gives a pink coloration with cotton parchment paper (after soaking in water) and a blue coloration with wood cellulose parchment paper (89).

According to Salvatirra and Noss (102), genuine and imitation parchment papers can be differentiated by the following two methods:

1. Examination of cross sections for secondary fluorescence when dyed with Rhodamin 6 GD.

2. Examination of cross sections mounted in paraffin oil with polarized light.

Differentiation Between Pasted Cartons and Index Bristols (103)

Since most cartons are pasted with starch, Faldner recommends the use of a dilute iodine solution for differentiating pasted cartons and index bristols. The sample is torn in such a manner that an oblique tearing surface, as wide as possible, results. The entire surface is stained with the solution, and any pasting will show up as dark blue lines. Starch sizing does not interfere with the test, because the darker lines are always clearly discernible. Index bristol, not being a laminated product, naturally gives no color.

Differentiation Between Pasted and Unpasted Boards (104)

A sharp cross section of the paper is prepared and the following tests are made: (1) Sodium silicate adhesive. The edge is moistened with 0.5% phenolphthalein in alcohol, allowed to dry, moistened with distilled water, and examined promptly. If sodium silicate is present, a deep red line will appear. (2) Starch adhesive. An iodine solution is used.

Watermark Identification (105)

Watermarks are manufactured by impressing the pattern on the paper. If the impression is effected on the wet-end of the paper machine or in case of a handmade paper on the wire cloth of the mold into the wet web of the paper, a natural watermark results. If, however, the impression is effected on the finished paper then one speaks of an artificial watermark.

If a paper with a natural watermark is immersed in a 28% solution of sodium hydroxide, after a very short time the watermark becomes more predominant and remains clearly visible even after lengthy soaking. If a paper with an artificial watermark is treated in the same way, the disappearance of this mark occurs after a short time. The reason for this disappearance lies in the swelling of the artificially pressed fibers during contact with the alkaline solution. During the impression of the pattern into the soft web, in the case of natural watermarks, the fibers are pressed in all directions, thus forming a thin place in the web. With the artificial patterns we are concerned with the compressed texture of the paper, which explains the different reaction.

Fluorescence Differentiation (106). Artificial watermarks produced by impression on the nearly dried paper with a rubber stamp are differentiated from genuine by sprinkling the area with a mixture of 100 g. of dry icing sugar and 0.5 g. of concentrated Rhodamin 6G, placing the paper in a dish of water, and examining in filtered ultraviolet light. The design of the genuine is marked for a few seconds by a bright golden fluorescence, which is absent from the artificial watermark.

Identification of Waxes (107)

Wax crystals are obtained by dissolving the wax in a suitable solvent at the solution temperature of the wax in question and allowing to cool spontaneously to room temperature. Although the waxes represent mixtures of several constituents, yet each wax will tend to produce just one or, at most, two types of crystal formations. Monoamylamine, due to its dual nature of solvent and chemical reagent, was found to be very suitable for wax crystallography. N-butyl alcohol was also used as a solvent. Some typical crystal formations were obtained with beeswax, carnauba wax, and ozokerite, when monoamylamine was used as a reagent. Odd types of crystal formations were obtained from potassium stearate in n-butyl alcohol and from cerotic acid in monoamylamine. In mixtures of waxes, some idea of the main constituents present may be obtained from the appearance of the crystal formations.

Identification of Printing

Occasionally, the pulp and paper microscopist is called upon to identify the printing process used, e.g., letterpress, offset, lithography, intaglio, or colotype. A detailed discussion of the problems involved in such identification seems to be beyond the scope of this book; however, the reader is directed to the following selected references (108-112).

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SPECK COUNT AND SPECK ANALYSIS

The relative freedom from foreign matter of pulp, paper, and paperboard is of technical, esthetic, and economic importance. It would seem, therefore, that methods used to determine the amount and kinds of dirt in pulp, paper, and paperboard ought to be performed in such a manner that the determination, both by the producer and the purchaser, will be comparable. Although there are many literature references to the analysis of specks, there are relatively few concerning systematic dirt count, and most of the methods given are subject to some question.

The main objections to these procedures are:

1. The so-called "Equivalent Black Area" depends both on the personal element in estimating the equivalent blackness of specks of different sizes, shapes, and colors, and on the ultimate use of the product under examination. A speck with a low "Equivalent Black Area" in many instances may be much more harmful than another type with a much larger value.

2. Limiting the size of the specks to be counted to 0.08 mm.^{2} "Equivalent Black Area" for pulp and 0.04 mm.^{2} "Equivalent Black Area" for paper is an arbitrary decision subject to question. In certain papers almost invisible specks can cause considerable harm and make the paper unsuitable for the use intended. Fortunately, T 437 ts-63, "Dirt in Paper and Paperboard," now includes a note which permits reporting separately the total "Equivalent Black Area" of specks between 0.01 and 0.04 mm.^{2} "if there are an unusual number of specks present."

3. The tentative standards ignore the counting of shives if they appear dark only at some particular angle of observation. Shives can, of course, cause considerable difficulty, particularly in printing papers. Happily, it is now suggested in T 437 that, if the number of shives present is noticeable, they should be recorded separately in the report, giving their approximate average actual area, their color or appearance, and the average number per square meter, for each side of the specimen.

4. One may ask of what value is it to know the relative "parts per million" of specks per unit area, or number of specks per gram of pulp, without knowing the type of speck present or predominant in the pulp or paper. First, it gives no opportunity for correcting the cause of the most predominant specks in the mill and, secondly, what may be very harmful specks in one kind of paper may be of little or no consequence in another kind.

Investigations by Adrian and Graff (1) indicated that for speck counts in pulp two methods should be used: (1) A laboratory method for the determination of the exact number of specks per gram of pulp, including speck analysis and the relative number of small, medium, and large specks of each kind; and (2) A mill control method for the determination of parts per million of unit area of small, medium, and large specks and a rough analysis of these. Graff (2) has found that for the speck count of paper practically the same method can be used as for the mill control method on pulp, with the exception that the use of reflected or transmitted light for the count depends upon the ultimate use of the paper.

SPECKS IN PULP

LABORATORY CONTROL METHOD (1)

In developing a laboratory control method which is reproducible and as free as possible from experimental and personal errors, the following points should be considered: Sampling; the method of preparing the handsheets – such as disintegration of the pulp, basis weight, and pressing of the sheets; the type of light box used – including type of illumination, distance of illumination from the sheet, and the height of the eyepoint above the sheet; the number of sheets to be counted for the least possible probable error in proportion to the time used; the frequency distribution of small, medium, and large specks in mm.^{2} of the specks; and the kind of specks predominant in each area.

A suggested procedure would be to use approximately 40 grams of air-dried pulp, or its equivalent of moist or wet stock thoroughly disintegrated in six liters of distilled water, care being taken that all equipment is absolutely clean. When the pulp has been thoroughly disintegrated, another six liters of distilled water are added to the container, the mixture is stirred with a glass rod, and 300 ml. are transferred to a 5.5-inch Büchner funnel fitted with a felt previously rinsed with distilled water. Eight sheets are prepared and placed one on top of the other but separated by the felts. The sheets are wrapped in a clean white paper or cloth, and pressed for two minutes under a one-ton over-all force. The sheets are then transferred to the speck counting box, where the number of specks is counted on a light box as shown in Fig. 123. This box is illuminated by two 20-watt fluorescent tubes with the top of the tubes placed 3.5 inches below the bottom of the ground glass plate. A ground-glass plate, smooth side up, is placed above the lights. A shield of heavy black paper, with a circular opening a little larger than the diameter of the sheet to be examined, is used to avoid the glare of the lights. A head or chin rest, 14 inches above the sheet, is used so that the eyes may always be a standard height above the sheets.

Each sheet in turn is placed over the ground glass plate of the light box, and each visible particle of foreign material is encircled with an indelible pencil. The sheet is then turned over and any particles visible from the other side of the sheet are encircled in the same way, and a note is made on the sheet of the total number of specks found. When each sheet of the set has been examined, the sheets are transferred to a drying oven, where they are hung and exposed to hot circulated air at 165°F. for 35 minutes, after which the dry sheets are weighed to the nearest tenth of a gram. The total number of specks found in all eight sheets, divided by the weight of the sheets in grams, equals the speck count per gram of pulp.

There is no reason why the Weyerhaeuser speck count box (Figure 124), which will be described under mill control method for speck counting, cannot be used for this purpose. However, both of these boxes should be equipped with 3 to 5 extra platforms 2, 4, 6, 8, and 10 inches high so as to adjust the chin rest to the height of the operator.

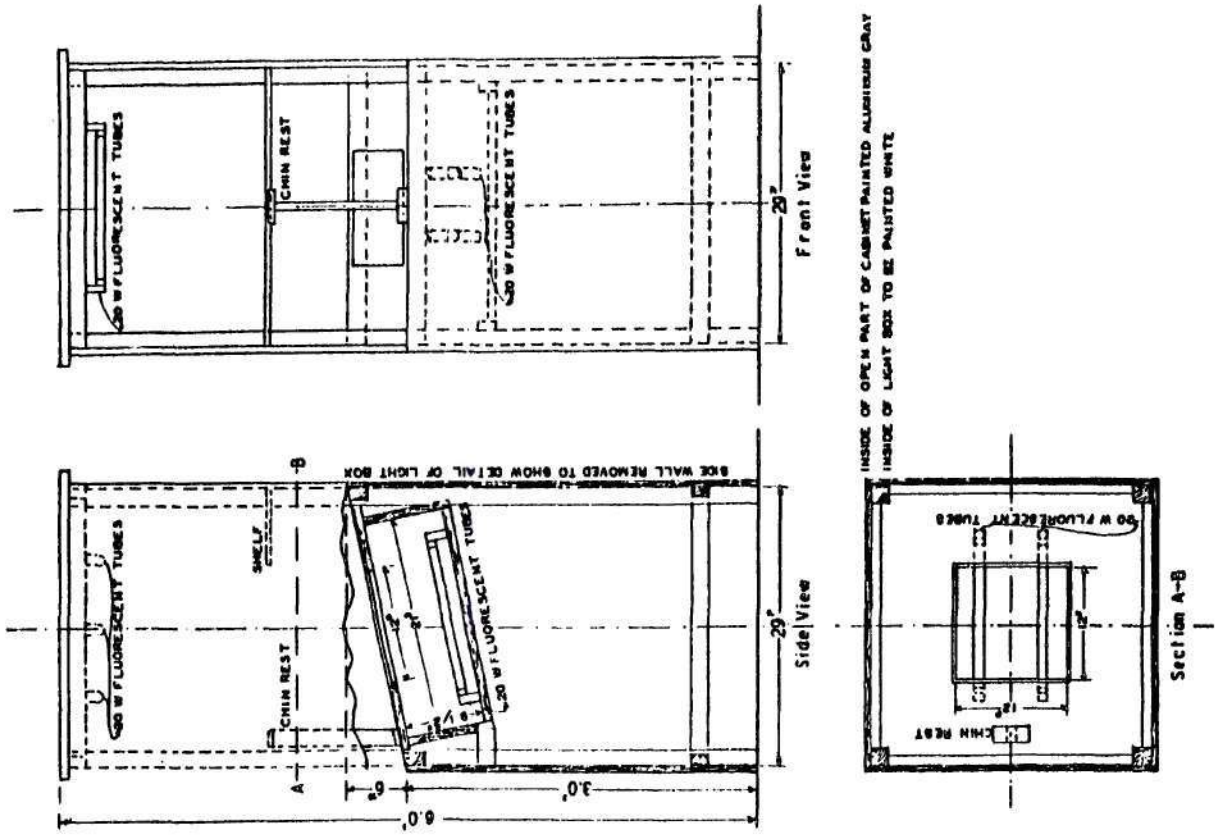


Figure 124. Weyerhaeuser Speck Count Box

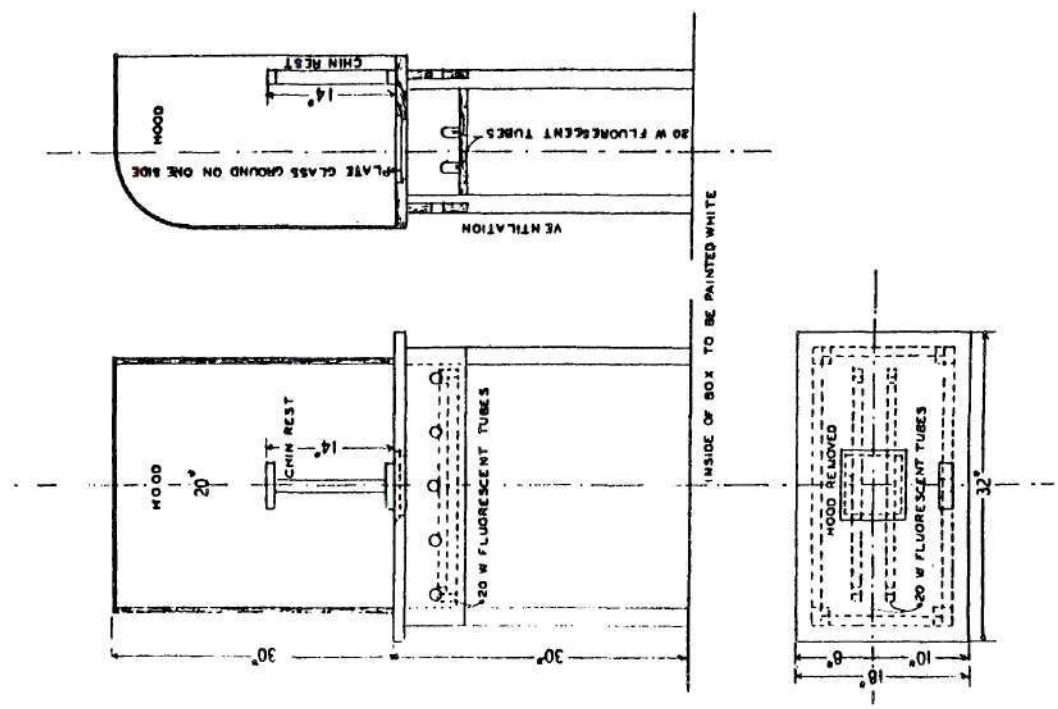


Figure 123. Laboratory Speck Count Box

Measurements and Analysis

From each of the dry sheets cut one or two 1 by 3-inch strips radially toward the center and place all of these strips in a large container where they can be thoroughly intermixed. Then, one strip at a time, rewet in distilled water containing 10% of glycerin, place on a 1 by 3-inch microscope slide, and place the slide on the microscope stage.

Using a 32-mm. objective and a 10X eyepiece with an eyepiece micrometer, the specks can be analyzed and the long and short diameter of the speck determined. Multiplying the long by the short diameter gives the approximate area of each speck. However, experience has shown that an exact measurement of the area of each individual speck not only is unnecessary but is quite time consuming. The foremost purpose of a significant determination is the relative number of small, medium, and large specks.

If we then consider as small specks those smaller than 0.05 $mm.^2$, medium specks those between 0.05 and 0.5 $mm.^2$, and large specks those larger than 0.5 $mm.^2$, small specks will include more or less circular specks with their diameter less than 0.252 mm., medium specks will be those whose diameter is between 0.252 mm. and 0.798 mm., and large specks those with a diameter greater than 0.798 mm. Rectangular or irregular-shaped specks with the same area limits as the circular specks would, of course, have variable dimensions.

Specks in wood pulp, as a rule, consist mostly of resins, gums, knotwood, inner and outer bark, and shives, all of which after some experience an operator can easily identify without the use of any reagent. All other specks, for practical purposes, may be classed under miscellaneous except when they are very prominent or considerably above the routine count in the mill. Each individual speck of this class must be treated as shown in the chart for speck analysis.

Report

If the 1 by 3-inch strips cut radially toward the center are thoroughly intermixed for good sampling, 50 to 100 specks, depending upon the accuracy desired and the time available, should be enough for a fairly accurate determination of the size and kind of specks in the pulp. If the speck count (number of specks per gram of pulp) should be extremely low, it will be necessary to cut three or more strips from each sheet.

The results for a sample of bleached sulfite pulp, for example, can be reported in any one of the three methods illustrated in Tables XXXIIA, XXXIIB, and XXXIIC. Each of these three methods of reporting the relative amount and kind of specks in pulp shows that, so far as this sample is concerned, the predominant specks are resins, inner bark, and shives. However, the resin count, although 38% of the total number, is relatively low, as it is not uncommon to find up to 60% resin specks in relatively clean bleached sulfite pulp.

On the other hand, the percentages by number of inner bark and shives, which are practically all medium-sized specks, indicate that this pulp could have been made considerably cleaner by more careful barking and by better screening facilities or screen operation.

Comparing the three methods, it is also seen that there is no particular advantage in determining the total area of the specks.

TABLE XXXIIA

PERCENTAGE CLASSIFICATION OF NUMBER OF SPECKS PER GRAM OF PULP

No. of Specks per g.	Resins, %	Knotwood, %	Inner Bark, %	Outer Bark, %	Shives, %	Felt Hairs, %	Misc., %
61	38	4	24	2	28	3	1

TABLE XXXIIB

PERCENT FREQUENCY AND TOTAL AREA OF SPECKS
(Measuring the area of each speck)

Specks	Frequency, %	Area, mm.^{2}	Area, %
Resins	38	2.782	9.07
Knotwood	4	2.351	7.66
Inner bark	24	4.006	13.05
Outer bark	2	0.870	2.83
Shives	28	20.550	66.90
Felt hairs	3	0.091	0.35
Miscellaneous	1	0.042	0.14

TABLE XXXIIC

PERCENT BY NUMBER OF SMALL, MEDIUM, AND LARGE SPECKS

Specks	Small, %	Medium, %	Large, %
Resins	16	22	--
Knotwood	--	4	--
Inner bark	3	21	--
Outer bark	--	2	--
Shives	2	26	--
Felt hairs	3	--	--
Miscellaneous	--	1	--

MILL CONTROL METHOD (1)

This method is to be used for continual checks during the manufacturing operation, and the number and kind of specks are determined directly from lap or drier sheets. This method must be reproducible, free from experimental errors and variation of personal judgment as far as possible, and so arranged that it can be carried out in a minumum of time.

The type of light box, kind and position of the illuminant, as well as the height of the eyepoint of the observer is also of importance. As in the laboratory method, the number of test sheets, the area and frequency distribution of small, medium, and large specks, and kind of specks in each group must be considered, although to a more limited degree.

The Light Box

The light box is a slight modification of the so-called Weyerhaeuser box, described by Adrian and Graff (1), which is illustrated in Figure 124. As stated under the description of the Laboratory Control Method, this box can also be used for speck counting of the handsheets and for speck count in paper.

Sampling

Based upon the experience gained from the study of the evaluation of the different methods of speck counts in paper (2), one or two sheets should be taken from the cutter box of the machine every hour when continuous control is needed, and this sample cut up into 8 1/2 by 11-inch sheets, and five of these sheets used for the speck count. If the speck count is only done for each shift, or once a day, an average set of sheets is taken from the one-hour samples.

Illumination

Experience gained by speck count in paper did show that if three overhead 20-watt daylight fluorescent lights were used for illumination, the total speck count was about 40% less than if only one 20-watt fluorescent light was used, and most of these specks were between zero and 0.05 mm.^{2}. Apparently, too much reflected light obscured a large number of the small, light-colored specks. Hence, for the bleached pulps, only one 20-watt daylight fluorescent light should be used. For unbleached pulps, however, there may be an advantage in using two bulbs.

Counting

It takes considerable time to count every speck on both sides of five 8 1/2 by 11-inch sheets, but a modification of Simmonds, Billington, and Bairds' sampling method (3) reduces this time considerably without increasing the probable error of the count.

A metal template, like the one shown in Figure 125, was designed to give selective sampling areas of 10 in.^{2}, which can be outlined with a pencil and, by turning the sample sheet on its long axis, ten sampling areas are obtained on the other side, without interfering with those on the first side.

Using the sampling areas of 20 in.^{2} of each sheet only, the total number per sq. meter of pulp sheets for each set of five sheets is obtained by multiplying the result by 30.86.

After the sampling areas have been marked, each sheet in turn is placed in the speck-counting box, and every speck observed in each encircled area on both sides of the sheet is counted and marked with a small circle.

When all the visible specks in the five sample sheets have been marked and counted, the total count is, as stated above, multiplied by 30.86 to get the number of specks per sq. meter of the pulp sheets. If desired, this may be converted to specks per pound of air-dried pulp, if the basis weight is known.

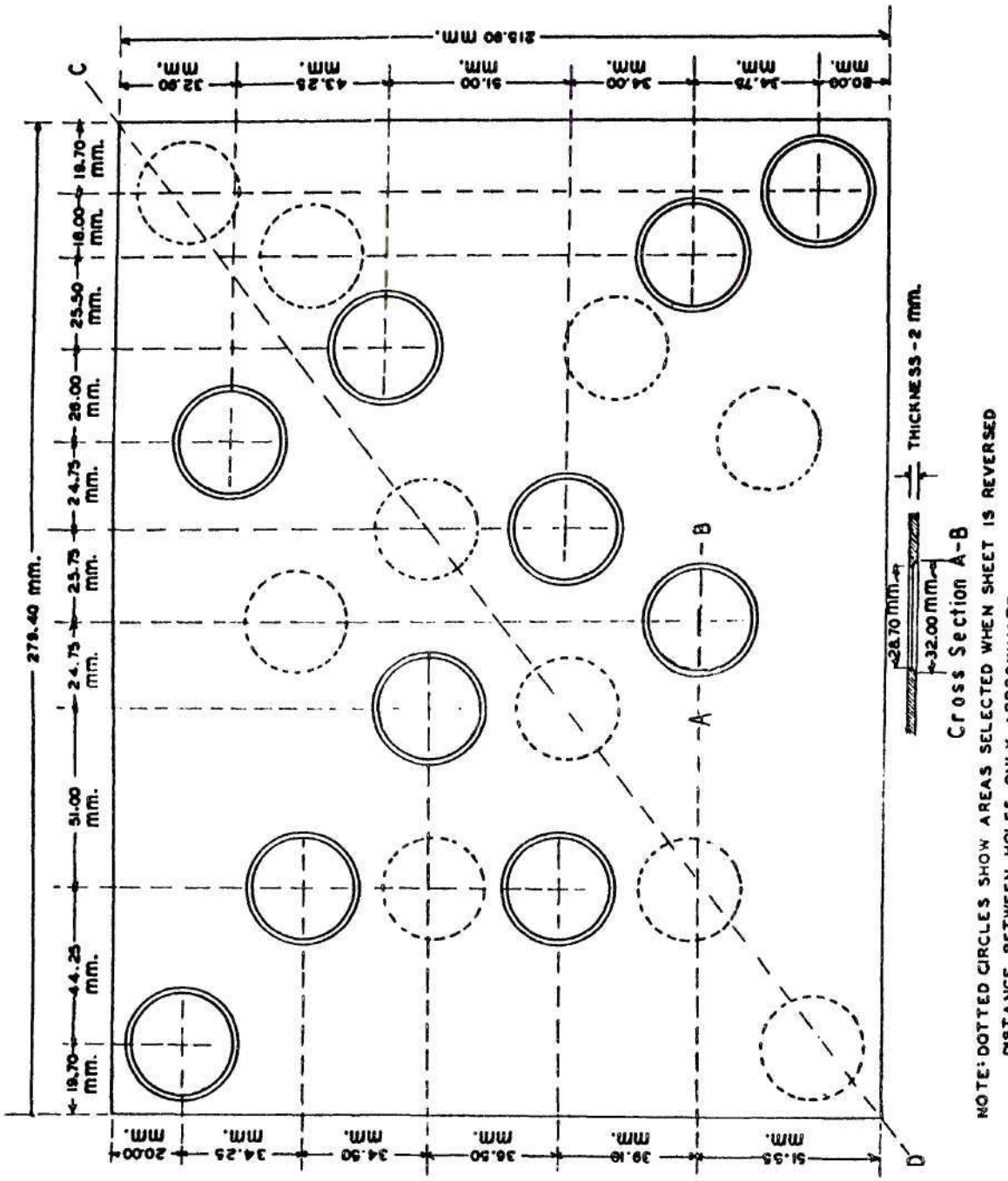


Figure 125. Template for Area Sampling for Speck Count

Measurements and Analysis

When all the specks have been marked, a few of the circular sampling areas are cut from each of the five sheets, placed in a large beaker, and thoroughly intermixed. One disk at a time is then removed from the beaker and placed, with the marked specks up, on a microscope slide and examined and measured microscopically under low power.

Measurements and analysis are performed in the same way as described for the Laboratory Control Method. That is, the areas of the specks are divided into small, medium, and large specks, and speck identifications are limited to those specks, in wood pulps, which can be recognized without the use of reagent. However, if an exceptionally large amount of unknown specks, for one reason or another, should be present, the analysis of these has to be done as shown in the Chart for Speck Analysis.

Report

Because of the thickness of the lap or drier sheet a large number of specks, unseen, is hidden between the two surfaces of the pulp sheet; therefore, it seems logical to report the number of visible specks per square meter of pulp sheets instead of by number of specks per pound of air-dried pulp; if properly sampled, 50 specks should be sufficient for measurements and analysis.

From a sample of unbleached sulfite pulp, for example, which gave 1945 specks per square meter, two different sets of measurements and analysis were made of 50 specks with the results shown in Tables XXXIIIA and XXXIIIB. Comparing the tabulated data of these two sets of 50 measurements and analysis, it is apparent that one set of 50 specks should be sufficient.

TABLE XXXIIIA

PERCENTAGE CLASSIFICATION OF NUMBER OF SPECKS PER M.^{2}

	No. of Specks per M.^{2}	Resin, %	Inner Bark, %	Outer Bark, %	Shives, %	Misc., %
Test 1	1945	42	34	8	4	12
Test 2	1945	44	32	6	4	14

The advantage of this method of tabulation is that it discloses the nature of the dirt or specks causing trouble from a sales viewpoint, the relative amount and size of the most objectionable specks, and also gives the mill operator an idea of the origin of the objectionable specks.

However, examination of the data shows that 14% by number of the total number of specks were miscellaneous, or specks unknown to the observer. Although all of these were small, special analysis should be made of them in case this count is above the usual count of these for this mill.

TABLE XXXIIIB

PERCENT BY NUMBER OF SMALL, MEDIUM, AND LARGE SPECKS

	Specks	Small, %	Medium, %	Large, %
Test 1	Resin	22	20	--
	Inner bark	4	30	--
	Outer bark	2	4	--
	Shives	--	4	--
	Miscellaneous	12	2	--
Test 2	Resin	28	16	--
	Inner bark	2	30	--
	Outer bark	--	6	--
	Shives	--	4	--
	Miscellaneous	12	2	--

The time required for counting, measuring, and classifying the speck is less than one hour, and the tabulation and reporting takes only a few minutes.

SPECK COUNTS IN PAPER (2)

The cleanliness of paper, as well as of pulps, depends, to a large extent, upon the number of specks and the total area of these specks in a given area of the paper. This so-called cleanliness also depends upon the ultimate use of the paper and whether the paper is examined under reflected or transmitted light. For example, for papers used for electrical or photographic purposes or for data cards for electrical classifying machines, metallic and other conducting specks, as cinders, are the most objectionable. The same is true for papers used with transmitted light, for lampshades or other decorative purposes, when the speck count must be done by transmitted light.

Time is also an important element here because with the many types of papers which go directly from the cutters to the sorting and wrapping table and then directly to the shipping room, information as to the relative cleanliness of the paper must be known as soon as possible.

The specifications considered necessary for the light box for the speck counting of pulp sheets and the height of the chin rest also are the same for paper, so that the modified Weyerhaeuser light box is also ideal for speck count in paper.

SAMPLING

Experiment has shown that, by proper selection of the paper samples, there is no practical difference between the average of the count per square meter for 5, 10, 15, 20, or 25 sheets for the test.

Former work has also shown that, using the sampling template as recommended for sampling areas of the pulp sheets, and as illustrated in Figure 125, there is no practical difference between counting all the specks on both sides of the five 8 1/2 by 11-inch sheets and multiplying the results by 3.3 to get the total number of specks per square meter and only counting the specks in the ten sampling areas on both sides of the five sheets and multiplying the results by 30.86, or by counting the specks in the ten sampling areas of the sheets alternately on the felt and wire side of the five sheets and multiplying by 61.72, or only counting the specks in the five sampling areas on any one of the sides of the line C-D in Figure 125 on the felt and wire side of the five sheets and multiplying the results by 123.44 to get the number of specks per square meter of the paper.

Equal consideration must, as far as possible, be given to the felt and wire side of the paper, as there generally are more specks on the felt side than on the wire side of the sheet.

COUNTING

When the sampling areas have been marked with the template on both sides of the 8 1/2 by 11-inch sheets, the sheets are placed singly in the speck counting box, illuminated with one overhead 20-watt daylight fluorescent light, every speck in the 20, 10, or 5 inch sampling areas marked and counted on both sides of the sheets, and the total number then multiplied by 30.86, 61.72, or 123.44, depending on the total sampling areas used, to get the total number of specks per square meter of paper.

If the speck count must be done by transmitted light, the sampling areas are not marked, but the sheets are laid one at a time directly on the light box, the sampling template placed directly on the top of the paper, and the specks visible through the openings in the template counted and marked. When the specks are marked on one side of the sheet, the sheet is turned around on its long axis under the template, and the specks in the areas under the template counted and marked in the same manner. The illumination for the transmitted light is by two 20-watt daylight fluorescent lights located as shown in Figure 124.

MEASUREMENTS AND ANALYSIS

When all the specks have been marked and counted, each marked speck is carefully cut out with a pair of small scissors and placed in a beaker where they are thoroughly intermixed. They are then taken, one at a time, with a pair of forceps, soaked in a 10% glycerin solution in distilled water and placed side by side on a microscope slide. When the desired number of specks has been placed on the slide, it is put in the mechanical stage of a microscope, which is equipped with a 32-mm. objective and a 10X eyepiece holding a micrometer scale.

The measurement of the specks and the classification into small, medium, and large sizes is done as before, but the analytical classification of the specks depends upon the papers themselves.

If the papers are wood pulp papers for speck analysis in the mill where they are made, the proposition, except in special cases, is about the same as the

Laboratory Control Method for speck analysis in the pulp mill, and 50 to 100 specks normally should be enough both for measurement and analysis.

If the papers are from another mill, from an unknown source, rag papers, or for the identification of special types of specks, a much more careful analytical procedure, like those described under the section for speck analysis, must be followed.

REPORT

For routine determination of the relative number of specks per square meter and the percentage classification of small, medium, and large specks, the method illustrated in Tables XXXIIIA and B may be used. When the purpose is to identify special types of specks or the relative amount of special specks in certain types of papers, as, for example, metallic specks in photographic or electrical papers, different methods must be used both for the analysis and the report.

SPECK ANALYSIS

Specks or dirt in pulp or paper can, as a general rule, be divided into three main groups (4):

1. Raw material dirt from wood, rags, or other cellulosic products, or from materials added during the manufacturing operation.

2. Process dirt.

3. Mill dirt, which can be subdivided into

   (a) Air-borne dirt, such as soot, coal, seed hairs, insects, etc.;

   (b) Water-borne dirt, such as pipe scale, slime, filter sludge, etc.;

   (c) General dirt, such as oil, grease, earth, etc.

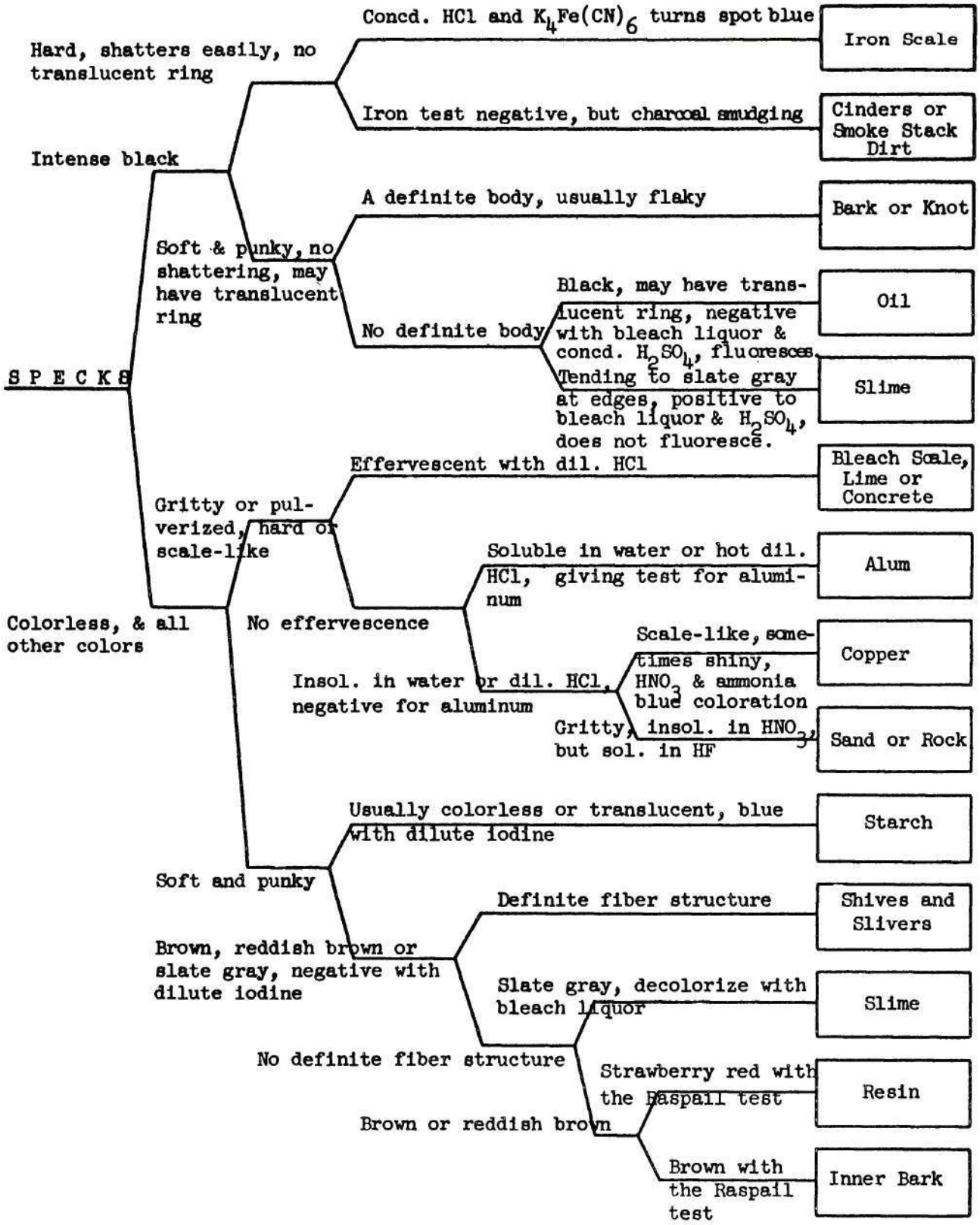
If an analysis is to be made of the specks in wood pulps (handsheets or lap sheets), the radially cut strips from the laboratory sheets or the circles with the marked specks of the lap sheets are, as previously described, cut out, thoroughly intermixed, dipped individually in a 10% glycerin solution, placed on a microscope slide, and examined under a microscope equipped with a 32-mm. objective and a 10X eyepiece, where measurements and analysis are made.

For a quick analysis of the specks in wood pulp lap sheets most of the specks can be recognized easily without reagents or stains, after some experience, but in special instances, when the analyst is uncertain, or specks not common to the average run of a particular mill are predominant, a dichotomous key (Table XXXIV) modified after the suggestions of Parkinson and Sherman (5) can be of considerable assistance for a quick determination of the type of specks present.

Another key, "The Systematic Identification of Spots and Specks in Paper," devised by Dalton and Wiltshire (6), as well as "Chart for Speck Analysis," published

TABLE XXXIV

KEY FOR THE IDENTIFICATION OF THE MORE COMMON FORMS OF DIRT IN PULP AND PAPER



by Browning and Graff (7), both describe more detailed analysis of the different specks and should be used not only for paper, but also for the more careful analysis of the specks in the laboratory handsheets for speck analysis of pulp.

If the analysis is concerned with the specks in paper, the marked specks are cut out with the scissors, thoroughly intermixed and, one at a time, soaked in a 10% glycerin solution. A number of these specks are then placed on a microscope slide and examined microscopically under low power, although in some instances a higher magnification may be required for best results.

If the speck cannot be identified when imbedded in the sheet, it is removed to a beaker of water for rinsing, and then, with the help of a low-power dissecting microscope, carefully removed from the sheet with iridium-platinum needles, placed on a slide and reexamined. A difficulty with this procedure is that the specks sometimes break up so that dimensions cannot be determined, although the analysis has become easier.

THE SYSTEMATIC IDENTIFICATION OF SPOTS AND SPECKS IN PAPER (6). TABLE XXXV

The specks are first divided into two groups:

I. Speck transparent or translucent with transmitted light.

II. Speck opaque with transmitted light.

The specks in each group are then subdivided as follows, according to their physical characteristics:

I. A. Soft, spreadable, tacky, or impregnated.

B. Soft, not spreadable, elastic.

C. Hard, friable, powdery.

D. Hard, fibrous.

E. Hard, cannot be crushed.

II. A. Soft, spreadable, tacky.

B. Soft, not spreadable.

C. Powdery, friable.

D. Hard, can be crushed.

E. Hard, cannot be crushed, metallic.

F. Hard, cannot be crushed, nonmetallic.

G. Fibrous – long extraneous fibers.

H. Fibrous – clots or lumps.

TABLE XXXV

THE SYSTEMATIC IDENTIFICATION OF SPOTS AND SPECKS IN PULP AND PAPER

Transparent or Translucent by Transmitted Light

Group	Physical Characteristics	Test	Result	Probable Identity	Confirmatory Test[a]
A	Soft, spreadable, tacky, impregnated, or an impregnated translucent halo	1. Treat with warm org. solvent, alcohol, ether, etc.	Spot disperses. A ring left around the edge	Oils, waxes, greases	Examine by Test 2.
		2. Filtered ultraviolet light	a. Blue fluorescence	Mineral oil, wax, grease	Compare with standard samples
			b. Yellow fluorescence	Vegetable oil, wax, grease	
B	Soft, not spreadable, elastic Usually with regular outline. Easily removed	1. Treat with drop of org. solvent	No reaction	Inconclusive	Examine by Test 2.
		2. Remove, heat gently over flame	a. Spot melts, burns readily, odor of burning rubber.	Rubber, i.e., rubber bands	Originate from waste paper
			b. Spot does not melt, burns, may give acrid vapors	Cellophane, transparent plastics	
C	Hard, friable, powdery, can readily be crushed	1. Treat spot with warm alc. or ether	a. Spot disperses. Ring around edge of drop. May be residue	Rosin	Examine by Test 2.
			b. Spot does not disperse much and is not softened	Pitch	Examine by Test 3.
	Spot usually colorless to amber color	2. Crush with a drop of acetic anhydride, then add a drop concd. H_2SO_4 Liebermann-Storch test	Fugitive violet color passing through red to gray-green	Rosin size spots	Compare with color given by rosin
	Spot usually golden to reddish brown	3. Apply Liebermann-Storch test given above	a. Fugitive violet or red color going to gray-green	Calcium or aluminum resinates	Can be tested for calcium or aluminum
			b. Fugitive red color going to yellow-brown	Natural resin from wood pulps	Compare with pitch from machine rolls
D	Hard, fibrous. Broken with needle give indistinct edge Colorless Any color Yellow to brown	1. Treat spot with a drop of org. solvent	No effect		Apply Test 2.
		2. Treat with drop of NaOH. Dry by warming.	Transparency is usually lost		Apply Test 3.
		3. Remove spot, break up with needle and stain with "C" Stain. Examine under the microscope	a. Very few fibers. Blue mass	Starch shiner	Treat spot with Iodine, turns blue
			b. Some fibers, fiber debris, clay, dirt, or filamentous growth	Slime spots (probably glazed in calender)	Color of spot may indicate source
			c. Tangled masses, clots, or strings of papermaking fibers	Pulp shiners, crushed in the calenders	
			d. Bundles or chips of raw fibers	Wood shiners. Shives glazed in calender	
E	Hard. Cannot be broken or powdered, not fibrous, usually a clear outline, easily removed from sheet	1. Treat spot with drop of org. solvent	No effect		Apply Test 2.
		2. Remove spot from sheet, stain with "C" Stain	Do not stain		Apply Test 3.
		3. Examine under microscope at x100	a. Spot is thin, flat, transparent, and colorless plates	Mica	Heat to redness, cool, and examine
			b. Spots colorless or yellowish crystals or round particles	Sand. quartz	As above

[a] Confirmatory test – see number in "Test" column for subdivision.

TABLE XXXV (Continued)

THE SYSTEMATIC IDENTIFICATION OF SPOTS AND SPECKS IN PULP AND PAPER

Opaque by Transmitted Light

Group	Physical Characteristics	Test	Result	Probable Identity	Confirmatory Test[a]
A	Soft, spreadable, tacky	1. Treat spot with drop of org. solvent (trichloroethylene is usually best)	a. Spot disperses forming ring around edge. Black residue	Grease. wax	Examine by Test 2. Residue by Test 3.
			b. No action		Apply Test 4.
		2. Examine original spot and ring formed by solvent under filtered ultraviolet light	a. Blue fluorescence	Mineral wax or grease	Compare with known samples
			b. Yellow fluorescence	Vegetable wax, grease, bituminous pitch	Compare with known samples
			c. Little or no fluorescence		Apply Test 4.
		3. Examine for lime or chalk, dirt or rosin			
		4. Remove from sheet. Heat over flame. Notice behavior, smell vapor, and test inflammability	a. Does not melt, odor of burning paint, burns, much ash	Soft paint	
			b. May melt, odor of burning rubber, burns	Soft rubber	
			c. Melts, burns, red residue left	Lipstick	
B.	Soft, not spreadable, may be elastic, flexible	1. Treat with org. solvent, i.e., trichloroethylene	No effect, or slight softening		Apply Test 2.
		2. Remove spot, place in crucible and heat, smell vapor, and test inflammability	a. Does not melt, odor of burning paint, burns, much ash	Paint	
			b. May melt, odor of burning rubber, burns easily	Rubber. gutta-percha	
			c. Does not melt, may shrivel up, acrid vapors, inflammable	Plastics	
C	Powdery, friable, with a "tail" or leaves a "tail" when rubbed White or off-white in color	1. Examine and classify into one of the three groups	a. White or off-white in color	Loadings	Examine by Test 2.
			b. Brightly colored spots or streaks	Dye or pigment spots	Examine by Test 5.
			c. Dark reddish-brown, brown or black	Inconclusive	Examine by Test 8.
		2. Remove, break up and stain with "C" Stain	May show few fibers, fine material, opaque powder, crystals	Loadings	Apply Test 3.
		3. Ignite, compare with ash from paper containing loading	Two ash are similar	Loadings	Apply Test 4.
		4. Introduce a little dil. HCl under the cover glass	a. Effervescence	Chalk, calcium carbonate, lime, bleach scale	
			b. Dissolves slowly	Calcium sulfate	
			c. No action	China clay, titanium dioxide	The ash can be tested for titanium
	Brightly colored spots or streaks	5. Treat the spot with hot water	a. Colored soln.	Dye spot	
			b. No action		Apply Test 6.
		6. Treat the spot with alc. and acetone	a. Colored soln.	Org. pigment	
			b. No action		Apply Test 7.
		7. Treat the spot with dil. HCl and potassium thiocyanate	Red coloration	Earth pigment. Prussian blue, etc.	
	Dark reddish-brown, brown or black	8. Examine under the microscope	a. Black, shiny		Apply Test 9.
			b. Black, or brown, dull		Apply Test 9.
			c. Reddish-rust colored		Apply Test 10.
		9. Remove from sheet, heat on crucible, smell vapors, see if spot will ignite	a. Brown smoke, tarry odor, yellow flame, little residue	Coal spots, crushed in calenders	
			b. Does not ignite, glows, very little residue	Coke spots	
			c. No change		Apply Test 10.
		10. Treat spot with dil. HCl and potassium thiocyanate solution	a. Intense red coloration	Rust, iron oxides, red and black	
			b. No color or faint color	Cinders, ashes. Dirt generally	

[a] Confirmatory test – see number in "Test" column for subdivision.

TABLE XXXV (Continued)

THE SYSTEMATIC IDENTIFICATION OF SPOTS AND SPECKS IN PULP AND PAPER

Opaque by Transmitted Light

Group	Physical Characteristics	Test	Result	Probable Identity	Confirmatory Test[a]
D	Hard, can be crushed. Dark brown, shiny	1. Treat spot with drops of org. solvents, ether, alcohol, etc.	May show extraction halo, spot softened	Pitch	Apply Test 2.
		2. Crush spot with a drop of acetic anhydride. Mix with a drop of concd. H_2SO_4	Fugitive red color, yellowish brown	Pitch from wood pulp	
	Black, shiny	3. Treat spot with drops of org. solvents	No effect		Apply Test 4.
		4. Burn spot on end of needle, smell vapors	Burns with yellow flame, brown smoke, tarry odor	Coal	
	Dark or colored flat plates	5. Treat spot with solvents	Softens in trichlorethylene	Paint	Usually flat plates, examined on end show definite layers. May be different colors.
		6. Burn on end of needle	Typical odor of burning paint	Paint	
	Brightly colored	7. Treat spot with solvents	May extract slightly		Apply Test 8.
		8. Heat gently on end of needle	Goes soft, plastic, burns with smoky flame	Sealing wax	From waste papers
E	Hard, cannot be powdered. Bright, obviously metallic, white	1. Treat spot with dil. acids	a. Dissolves		Apply Test 2.
			b. Insol., or leaves a residue		Apply Test 3.
		2. Treat soln. with $K_4Fe(CN)_6$ soln.	a. Blue-green precipitate or color	Iron	Thiocyanate or dil. HCl on spot
			b. White precipitate	Zinc	
		3. If spot insol. or a white residue is left examine original spots	a. Hard, brittle	Tin	
			b. Soft	Lead, solder	
	Yellow or reddish-brown	4. Treat with dil. acids	a. Sol.		Apply Test 5.
			b. Partly sol. White residue		Apply Test 6.
		5. Treat soln. with $K_4Fe(CN)_6$ soln.	Brown precipitate	Copper	
		6. If a white residue test soln. as in 5	Brown precipitate	Tin, copper, brass, bronze	
F	Hard, cannot be powdered. Not metallic	1. Treat with dil. acids	No effect		Apply Test 2.
		2. Examine under the microscope	Transparent or opaque crystals	Sand, quartz, etc.	Compare with samples of sand
G	Fibrous – long extraneous fibers	1. Remove fiber from paper and examine on a slide. Stain with "C" Stain	May be natural fibers (raw) or synthetic fibers	Hair, vegetable fibers, e.g., bristles, sisal, hemp, jute, wool, rayon, asbestos, etc.	Compare with illustrations in textbooks and with known samples
H	Fibrous – clots or lumps	1. Treat with alc.	a. Spot softens or disperses	Size spots	Apply Liebermann-Storch Test
			b. No effect		Apply Test 2.
		2. If spot is light in color and opaque ignite, and compare residue with the ash of a piece of paper	Residue similar to normal ash, may be coarser	Clots of scum, etc., from breast box	
		3. Spot should be removed from sheet as cleanly as possible, place on slide, break up, stain with "C" Stain	Very few fibers, blue mass	Starch specks	Apply iodine soln. to original spot
			Few fibers, much fiber debris, clay, etc., may show filamentous growths	Slime spots	
			Bundles or chips of raw or partly cooked fibers	Shives, sawdust, etc.	
			Tangled masses, clots of paper-making fibers	Fiber clots, broke spots, Oxidized mechanical pulp, paper, etc.	

[a] Confirmatory test – see number in "Test" column for subdivision.

When the speck has been classified into one or more of these subdivisions, various tests are suggested in the table which will lead to the probable identity of the speck. Confirmatory tests are also given.

CHART FOR SPECK ANALYSIS (7). TABLE XXXVI

This chart shows the physical properties and some of the solubility tests for the most common specks. The solubility tests and the chemical tests are referred to by number, and are explained in the following text. Only tests which may be applied directly and which do not involve chemical separations have been listed.

Solubility Tests

1. Dilute hydrochloric acid (3N) is a solvent for iron, rust, pipe scale, and alum. Alumina and gypsum specks dissolve when heated, and bleach scale and some button specks effervesce. Manganese dioxide specks dissolve in concentrated hydrochloric acid.

2. Eighty percent sulfuric acid: Outer and inner bark, knotwood, and shives are only partly soluble, but swell and become brown with this reagent. Resin and pitch dissolve, with a yellow-brown to brown color reaction; rosin size spots dissolve slowly with an orange-brown color reaction for plain rosin size and rosin-clay size spots; rosin-alum size spots assume a yellow color. The reagent is a solvent for fiber knots and slime. Starch, bleach scale, alumina spots, rust, and pipe scale dissolve better when heated.

3. Five percent sodium hydroxide is a good solvent for resin, pitch, and glue. Casein dissolves slowly, and felt hairs swell when heated. However, 10% potassium hydroxide is a better reagent for felt hairs.

4. Alcohol is a solvent for some dye particles. Resin and pitch dissolve when heated, and oil and grease dissolve slightly.

5. Water dissolves alum specks and some dye particles. Glue dissolves when heated.

6. Ten percent nitric acid is a solvent for copper oxide and copper sulfite, and for lead specks when used hot. Concentrated nitric acid must be used for copper sulfide and is also better for metallic copper.

7. Benzene is a solvent for oil and grease; resin and pitch dissolve slowly, and unvulcanized rubber specks swell and dissolve only partly.

8. Alcohol containing one percent of concentrated hydrochloric acid is a good solvent for resin, pitch, and some dye particles, as well as for rosin size spots, although the solution of these spots may be slow in the case of mixtures.

9. Alcohol containing one percent of concentrated ammonium hydroxide is a solvent for some dyes.

10. Hydrofluoric acid is a solvent for silicate materials. It is usually necessary to warm the mixture, especially if the particles are very large, and solution may be somewhat slow. The reaction should be carried out only in the

TABLE XXXVI

CHART FOR SPECK ANALYSIS

		Physical Properties or State								Solubility Test					
	Kinds of Specks	Color (By Reflected Light)	Color (By Transmitted Light)	Hardness	Friability	Structure	Smudging or Streak	Melting Properties or Odor	Fluorescence	Dilute HCl 3N 1.	80% H_2SO_4 2.	5% NaOH 3.	Alcohol 4.	Other Solubility Tests	Chemical Test
Raw Material Dirt — From Wood	Outer bark	Black to brown	Yellow to brown	Soft	Crumbles	Scale & fiber			Brown		Partly Brown color				2,3 & 6
	Inner bark	Orange to brown	Yellow to brown	Soft		Fiber			Brown		Partly Brown color				2,3,5 & 6
	Knotwood	Yellow to brown	Yellow to brown	Hard	Splits	Fiber			Yellow		Partly Brown color				2,3,5 & 6
	Shives	Yellow to colorless	Translucent	Soft	Splits	Fiber			Purplish to Yellow-White		Partly Brown color				2,3 & 6
	Resin and pitch	Reddish brown, amber to colorless	Translucent	Soft	Punky	Flaky		Melt. Odor	Green-Yellow		Dissolve. Color Yellow-Brown	Dissolve	Dissolve (Warm)	7 & 8	5
From Rags	Rubber specks	Dark red to colorless	Dark to light yellow or red	Elastic				Odor	Mustard Yellow					7	
	Button specks	Colored to colorless	Opaque	Hard	Gritty	Scalelike				May Effervesce					
	Sealing wax	Any color	Opaque	Hard	Gritty			Melt. Odor	Deep Orange						
From Materials Added	Alum specks	Gray to colorless	Dark		Shatter	Scalelike			Dark violet	Dissolve (Warm)				5	11
	Rosin size spots	Amber to colorless	Translucent	Soft	Shatter	Round		Odor	Bright Yellow		Slowly. Yellow to Brown Color			8	5
	Starch spots	Gray to colorless	Translucent	Soft	Punky				Bluish white		Dissolve (Warm)				1
	Glue spots	Yellow or colorless	Translucent	Soft	Punky	Flaky		Odor	White			Dissolve		5	13
	Casein	Gray to colorless	Some translucent	Soft	Punky	Flaky		Odor	White			Slowly			4
	Filler spots	Gray to white	Opaque	Soft	Crumbles				Mustard Yellow						
	Dye particles	Color or dye		Soft									May Dissolve	5,8 & 9	
Process Dirt	Fiber knots	Colored to colorless	Translucent	Soft		Fiber					Dissolve				
	Felt hairs	Colored to colorless	Dark	Soft		Fiber						Swells (Warm)			
	Asbestos fibers	Gray to white	Translucent to opaque	Hard	Splits	Fiber			Purple						14
	Rubber specks	Dark red to colorless	Dark to light-yellow or red	Elastic				Odor	Mustard Yellow					7	
	Iron	Black to silver gray	Opaque	Hard						Dissolve (Rapidly)	Dissolve (Rapidly)				8,9 & 10
	Rust	Black to brown-red	Opaque	Medium	Shatters or crumbles	Scalelike or granular	Brown			Dissolve	Dissolve (Warm)				9 & 10
	Scale from mill pipes	Gray to brown-red	Opaque	Medium	Shatters or crumbles	Scalelike with fibers				Dissolve	Dissolve (Warm)				9 & 10
	Copper sulfide	Black	Opaque			Fern Structure	Black							6	7
	Copper alloys	Metallic luster	Opaque	Hard	Scalelike chips or fragments									6	8,9 & 10
	Lead specks	Gray	Opaque	Medium			Metallic							6	
	Graphite	Black and shiny	Opaque	Soft	Greasy	Flaky	Gray to black		Deep purple						15
	Bleach scale, lime or concrete	Gray to colorless	Opaque	Hard	Gritty	Scalelike			Mustard Yellow	Effervesce	Dissolve (Warm)				
	Sand, silica, glass or tile	Colored to colorless	Opaque to translucent	Hard	Gritty				Purple					10	
	Alumina	Gray to colorless								Dissolve	Dissolve (Warm)				11
	Gypsum	Gray to colorless		Medium	Shatters	May have crystals				Dissolve (Warm)					
	Manganese dioxide	Brown to black	Opaque		Gritty		Brown			Dissolve in Concentrated				11	
	Foam spots	Yellow brown to colorless	Opaque translucent rings	Soft		Circular									
Mill Dirt	Coal and carbon	Black	Opaque	Medium	Shatters	Conchoidal	Black								
	Cinders	Black	Opaque	Soft to hard	Shatters	Scalelike to porous									
	Oil or grease	Dark to light	Translucent	Soft					Blue to yellow				Dissolve slightly	7	
	Slime	Brown to colorless	Translucent	Soft		Stringy or flaky					Dissolve				6 & 12

absence of glass, for example, on a celluloid or bakelite slide, in a platinum crucible, on a platinum foil, or on a piece of wood. Microscopic observations should be made with as long a focal length objective as possible and only after suitable protection with a cover glass attached to the objective with a drop of glycerin or cedar oil.

11. Ten percent sodium metabisulfite is a solvent for manganese dioxide specks.

Chemical Tests

1. One-hundredth N iodine solution. The reagent consists of 1.3 g. of resublimed iodine and 1.6 g. of potassium iodide per liter of water. Starch is stained a deep blue and dextrin a wine red with a small drop of the solution.

2. Any of the iodine-iodide metallic salt stains used for fiber identification. Add a drop of the stain to the speck on the slide, put on a cover glass, let stand for one minute, drain off the surplus, and examine under the microscope. This will show whether or not the speck is a cellulosic material; the color reaction with shives will vary according to the degree of purification and the type of stain selected, from yellow to gray violet to violet purple.

3. Phloroglucinol solution. The reagent consists of 2 ml. of a 5% alcoholic solution of phloroglucinol mixed with 1 ml. of concentrated hydrochloric acid before use. Add a drop of the solution to the speck on the slide, let stand for one to two minutes, drain off the surplus, and examine under the microscope. Lignified tissues are stained a purplish red. For outer and inner bark and knotwood, the reaction is slow and the color develops in spots. Shives from mechanical pulp are stained much darker than those from chemical pulp, and the color reaction depends upon the degree of delignification of the shive.

4. Millon's reagent. Dissolve 5 g. of mercury in 6.5 ml. of concentrated nitric acid, and dilute to 45 ml. in water. Add a drop of reagent to the speck on the corner of a slide and warm gently over a microflame. Casein gives a brick-red color.

5. Raspail test. Moisten the speck with a strong solution of sugar, remove the excess with filter paper, and add a drop of concentrated sulfuric acid. Alum-rosin size spots produce a raspberry-red color; inner bark, knotwood, resin, and pitch are colored a brown-red; and plain rosin and rosin-clay show a red-brown color. Rosin size spots give a much stronger reaction with this test than rosin-sized papers.

6. Bleaching powder solution. A clear solution of calcium hypochlorite containing about 5% available chlorine (made by adding one part of bleaching powder to seven parts of water, and filtering) may be kept in a stoppered amber bottle away from light for a long time. When used, the solution is acidulated with acetic acid by adding one drop of glacial acetic acid to 15 drops of the bleaching solution. A drop or two of this solution will decolorize bark, knotwood, shives, and slime specks. However, the reaction is very slow.

7. Ten percent potassium cyanide. If the speck is copper sulfide (dendritic growths) it will, when treated with a drop of this reagent, first become colorless and then gradually develop to a bright-yellow color. [Potassium cyanide is very poisonous. Do not add acid to the solution, because hydrocyanic acid is formed.]

8. Concentrated nitric acid and ammonia. Add a very small drop of concentrated nitric acid to the speck, warm the slide, and make slightly alkaline with ammonia. Copper compounds produce the deep blue copper-ammonia complex. Iron yields red ferric hydroxide; rust and pipe scale do not react with this reagent.

9. Ferrocyanide test. Warm the speck on the slide with a drop of 10% hydrochloric acid and add a drop of 2% potassium ferrocyanide. Rust and pipe scales yield the blue ferrocyanide, iron specks give a weak reaction with this reagent, and copper specks yield the brown ferrocyanide, but for copper, a concentrated solution of nitric acid is better.

10. Ferricyanide test. To the speck on the slide add a drop of 10% hydrochloric acid and then a drop of 2% potassium ferricyanide. Copper alloys yield brown ferricyanide, and iron specks the blue ferricyanide. Rust and pipe scale may give a blue color test if iron is present under the layer of oxide.

11. Potassium acid sulfate. To test for alum spots in material which is soluble in water, add a grain of solid potassium acid sulfate to the solution and allow the drop to evaporate slowly. Characteristic octahedral crystals of potassium alum separate. The test may be applied to alumina specks by dissolving in hydrochloric acid and adding potassium acid sulfate.

12. India ink smear test. Slime-forming bacteria can usually be detected in samples of slime stock if an India ink smear of the latter is stained and examined under an oil immersion lens. The stained smear is prepared as follows: Place a small quantity of the slime sample near one end of the clean slide. Add an equal volume of India ink and mix thoroughly by means of a clean needle or glass rod. Spread the mixed sample evenly by means of a second glass slide and allow to dry. The color should be grayish rather than black. Fix the dried film by passing the glass slide (film side down) two or three times through a hot gas flame. Then stain the fixed film for 45 seconds in 0.1% aqueous carbolfuchsin, after which wash off the stain carefully and allow to dry. Slime-forming bacteria have well-defined capsules which appear unstained but sharply outlined against the India ink background. Inside the capsules will be found pinkish-stained bacteria cells. It should be noted that it is difficult to identify slime in paper that has passed over driers or calenders.

13. Biuret test. Proteins treated first with a solution of copper sulfate and then with a sodium or potassium hydroxide solution give red and violet colors. An important aspect of this test is that it gives a fully negative result with rosin size. The speck on the slide is wetted with 2% copper sulfate solution, and after 4 or 5 minutes a drop of 5 to 10% solution of alkali is added from the end of a glass rod. The violet shows best after the slide has been drained by setting on edge.

14. Asbestos fibers are unchanged by ignition and are inert to all common reagents. They are decomposed by fusion with sodium carbonate.

15. Graphite is inert to almost all reagents. It burns almost completely on ignition, with loss of black color.

DETERMINATION OF METALLIC SPECKS IN PHOTOGRAPHIC AND ELECTRICAL PAPERS

These papers must be as free as possible from even the least traces of any type of metallic specks. Many of these specks may be invisible to the naked eye before treatment; thus, the number of such specks per unit area of the paper must be determined by cutting the samples into one square decimeter sheets and treating them in a large Petri dish by one of the following methods:

1. The paper is saturated with dilute (6N) hydrochloric acid to transform the iron into chloride. It is then soaked in a solution of potassium ferrocyanide. Each iron particle appears as a blue spot. After drying, a quantitative estimation may be made by counting the spots per unit area (8).

2. The paper is soaked in a 10% silver nitrate solution. The particles show up as black spots (8).

3. The paper is treated with 1 ml. of 0.1% benzidine or o-tolidine in alcohol and 25 ml. of the MacIlvaine buffer solution (pH 8) for one to two minutes, and, after washing with water, is treated with 30% ammonium thiocyanate. The appearance of blue color indicates the presence of copper or iron (9).

4. The sheet is soaked with 10% nitric acid solution and the acid allowed to evaporate at room temperature. It is then soaked in 1% potassium ferrocyanide solution, washed thoroughly with water, and the excess water squeezed out. Copper is detected as the reddish-brown copper ferrocyanide and iron as Prussian blue. This method is so sensitive that even specks which were previously invisible can be seen distinctly. The copper specks which can be detected in this manner consist mainly of the oxide, the sulfite, or metallic copper. Copper sulfide cannot be determined in this way, because it is insoluble in 10% nitric acid. For this, concentrated nitric acid must be used and the excess of acid diluted before treating the sample further as in the test for other copper salts (10).

5. The paper is steeped in a 1% acetic acid solution and thoroughly dried. It is then introduced into a solution containing 1 ml. of glacial acetic acid and 1 g. of potassium ferricyanide per liter. The particles of iron show up as blue spots (Turnbull's blue), and the copper spots are brown (8).

SUMMARY

Many of the most common specks found in pulp and paper are described in all three tables (XXXIV, XXXV, and XXXVI), some of the specks are only described in one or two of the tables, and some are often identified with another name or classification and may, therefore, not easily be found in the tables.

Many of the specks are also very similar or may contain some of the basic material of other specks. Therefore, to minimize error in the determination, the specks have been arranged into 22 different groups and the outstanding differences within each group have been more fully described. The analyst may also wish to consult TAPPI method T 445, "Identification of Specks and Spots in Paper," for helpful hints and additional references.

As previously mentioned, the specks described in the three tables, and in this summary, are only those identifiable by direct application of the tests given, and do not require chemical separation. Many others, including specks where chemical separation is necessary for identification, are often a problem for the analytical chemist familiar both with chemical microscopy and microchemistry (16-18).

GROUP I. ALGAE, BACTERIA, SLIME, ETC.

Foam or Froth Spots

In addition to information in Tables XXXV and XXXVI, these spots are recognizable by the fact that they contain fibers, that they are irregular in shape and differ from broke spots by being dark and more opaque than the surrounding paper when viewed with transmitted light (4).

Green Algae

These are water-borne specks, filamentous and similar in appearance to slime and bacteria, but green in color. They are usually recognized under the microscope and give a strong iron reaction, and frequently contain diatoms yielding characteristic forms after ignition (4).

Iron Bacteria

Slime, brown in color, and exhibiting a fine, feathery structure. There is a strong test for iron, and the characteristic form of the bacterial filaments is easily recognized under high power (4).

Mycelium Filaments

These are stock spots of various colored (mostly dirty olive green to dark brown) mycelium filaments and hyphae. Detection is made microscopically after defibering the spot in chloral hydrate and boiling the preparation under the cover glass. Even with medium magnification the fungus threads and spores stand out so clearly by their dark color that they cannot be overlooked. In many cases structural changes of the fibers are observed, which can be referred to the destruction of the cell wall (11).

Slime

Slime is described in all three tables. Fluorescence from it may be black or light-colored. The spot is soft and punky, has no definite structure, and will decolorize with bleach liquor. In general, the spot includes only a few fibers, but much fiber debris, clay, etc., and may have filamentous growths. Fritz (12, 13) states that the bulk of the slime is usually composed of filamentous fungi of the mold type. These form a matted tangle, in which bacteria, yeast, algae, and unicellular animals are frequently found. Bacteria and yeasts are the chief components in most cases.

Slime spots may often be identified by staining with ninhydrin (14), which gives a violet or blue color reaction to the protein which is always present in microorganisms.

Resinous Iron Specks

Another type of speck, which the inexperienced analyst may mistake for specks under this classification, is known as resinous iron specks. These may vary from translucent to opaque, from faint yellow to reddish-brown to black in color, are usually soft and punky, and have a resinous body saturated with iron salts.

GROUP II. ANIMAL FIBERS, WOOL, AND SILK

These fibers have been discussed in earlier chapters (VI and IX) and should present little difficulty for the analyst.

GROUP III. BARK AND KNOTWOOD

Outer and inner bark as well as decayed wood can be identified from Table XXXVI. Another type of speck in this group is phloem fiber.

GROUP IV. BLEACH SCALE, CONCRETE, LIME, ETC.

This group includes specks from bleach residue, bleach scale, calcium carbonate, calcium sulfate, concrete, gypsum, and lime. The methods of identification indicated in all three tables show that all of these types are practically the same except that the bleach scale and gypsum can be separated from the others because of their tendency to dissolve in warm dilute hydrochloric acid.

The article by Koch, Brandon, and Geohegan (15) as well as TAPPI method T 488, "Microscopical Identification of Fillers in Paper," will be helpful in the detection of some of these calcium compounds.

GROUP V. BUTTON SPECKS AND SEALING WAX

The most outstanding difference between these two types of specks is that the sealing wax melts, flows, and burns with a smoky flame and gives a strong odor.

GROUP VI. CASEIN, GLUE, ROSIN, AND STARCH

This group includes casein, glue, resinate spots (calcium or alum), rosin size spots, starch spots, and starch shiners, all of which can be found in Tables XXXIV, XXXV, and XXXVI. Although these look more or less similar, they can easily be separated by using one of the following reagents – Millon's, Raspail, or diluted iodine stain.

GROUP VII. HURDS, SHIVES, ETC.

This group consists of hurds, esparto scale, sclerenchyma specks, shives, wood, and wood shiners.

Hurds

These are incompletely separated woody residues of flax, hemp, or other herbaceous dicotyledonous plants (11).

Esparto Scale

This is dark brown in color and yields epidermal cells and characteristic tear drop cells (trichomes) on dissection.

Sclerenchyma specks in straw papers consist of knotlike aggregations of silicified sclerenchyma cells of cereal straws. Remove the knots from the paper with a dissecting needle and immerse in a drop of chloral hydrate on a slide. After covering with a cover glass, heat gently and then exert pressure on the cover glass to separate the cells in the clump. Oblique illumination will emphasize the glassy transparent cells against the background. After calendering of the paper, knots appear mostly as transparent spots.

The remaining types of specks in this group (shives, wood, and wood shiners) can easily be identified from the tables. Reference to details in Chapter VI will be helpful.

GROUP VIII. CINDERS, COAL, AND SOOT

The spots in this group are ash, carbon, coke, coal, graphite, and soot. They may be grouped as follows: (a) Ash and cinders; (b) Carbon, charcoal, and coal; (c) Coke; (d) Graphite; and (e) Soot, which is friable, resistant to chemical action and destroyed by ignition.

GROUP IX. DYES AND PIGMENTS, ETC.

Dye particles, soil particles, organic pigments, and pigment dyes are included in this group. With directions in Table XXXV (IIC), we find methods whereby dye particles, organic pigments, and pigment dyes can be separated from each other and earth pigments or dirt can be identified as dark, irregular spots, black or brown in color, gritty, and usually showing presence of sand after ignition.

GROUP X. FIBERS AND FIBER KNOTS

This group includes asbestos fibers, beard fibers, broke spots, fiber knots, pulp shiners, silk fibers, synthetic fibers, and various vegetable fibers, such as bast fibers, leaf fibers, grass fibers, etc. (see Chapter VI).

Asbestos

These fibers are best identified by the method in Table XXXVI.

Beard Fibers

These are various cellular impurities from cotton waste, generally brown or black in color. They are fragments of cotton hulls with the "beard fibers" attached principally to the narrow end of the seed and residue of leaves, stems, knots, etc. (11).

Broke Spots, Fiber Knots, and Pulp Shiners

These cannot be separated easily from each other except that broke spots tend to uniform structure, generally thin in the center and ring shaped (4).

Silk, synthetic, and vegetable fibers are best identified by comparing with authentic samples, photomicrographs, stain reactions, and, in some cases, with fiber cross sections (Chapter VI).

GROUP XI. FILLERS AND LOADINGS

Barium sulfate, calcium carbonate, calcium sulfate, chalk, China clay, diatomaceous earth, gypsum, talc, and titanium dioxide are in this group.

Calcium carbonate effervesces with dilute hydrochloric acid. Calcium sulfate dissolves in dilute hydrochloric acid; upon evaporation to dryness and addition of a drop of water characteristic crystals form. Barytes and talc are recognized by their regular and angular shape and by being almost transparent and, after ignition, by their characteristic forms. Diatoms are nearly pure silica and are recognized by their shapes and forms since they are fossil remains of microscopic plants. China clay and titanium dioxide do not react with dilute hydrochloric acid. The presence of titanium can be detected by special tests.

For assistance in the detection of mineral fillers, the analyst should refer to TAPPI method T 488 and to the article by Koch, Brandon, and Geohegan (15). Both of these references have photomicrographs of various crystals mentioned above.

GROUP XII. GLASS, QUARTZ, SAND, TILE, ETC.

This group includes glass, quartz, rock, sand, silica, and tile, which look similar and cannot be separately identified. These all dissolve in hydrofluoric acid, but only after prolonged treatment.

GROUP XIII. GREASE, OIL, AND WAX

All grease, oil, and wax specks dissolve in organic solvents, and the original spots or the rings around the dissolved spots fluoresce blue for mineral origin and yellow for vegetable origin, although this should always be checked with known samples.

GROUP XIV. INKS, LIPSTICK, AND PAINTS

Lipstick

These spots are soft and spreadable. When ignited in a crucible they melt, burn, and leave a colored residue.

Paint

These specks or spots can be separated easily into hard, flaky particles and soft or fresh paint. The flakes are soft but not spreadable as against the fresh paint which spreads easily. None of these specks melt but, if ignited, burn with odor of paint and leave much ash.

Printer's Ink

This may adhere to the fibers of repulped papers. If paper made from such stock is mounted in Canada balsam, the dark residue of the ink can be seen clearly (11).

GROUP XV. INORGANIC SALTS AND OXIDES

Alum, alumina, copper sulfide (dendritic growths), ferric chloride, and manganese dioxide belong to this group. The methods given in Table XXXVI may be used for identification.

Ferric Chloride

The large gray-black spots sometimes found on the drier sheets of bleached sulfite pulps may be ferric chloride surrounding iron specks. These stains or spots generally disappear in water. The following tests may be applied: (1) Evaporate the water extract and compare the residue with a known crystal of ferric chloride. (2) Treat the aqueous extract with silver nitrate solution; a precipitate indicates ferric chloride; if no precipitate is formed, ferric sulfate may be present.

Manganese Dioxide

These specks can be identified by treatment with 10% sodium metabisulfite solution. If the specks disappear, they are manganese dioxide.

GROUP XVI. IRON AND RUST

This group includes iron, iron oxide, iron scale, magnetic oxide of iron, pipe scale, and rust. There are not many differences among these specks except probably their color by reflected light, the strong reaction of pure iron specks with potassium ferricyanide, and the fact that pipe scale may have fibers attached to it.

GROUP XVII. NONFERROUS METALS

Brass, bronze, copper, lead, solder, tin and zinc specks are in this group. According to the three tables, brass, bronze, and copper react similarly and cannot be separated.

In Table XXXV lead and solder specks give the same results with the exception that the original lead specks give a more distinct metallic smear than the solder specks, and if the lead specks are dissolved in concentrated hydrochloric acid and the solution diluted, the precipitate can be compared with samples of lead chloride.

Tin responds to the same test as lead and solder but the original specks of tin are hard and brittle instead of soft. Zinc specks form a white precipitate when treated with dilute acids to which potassium ferrocyanide is added.

GROUP XVIII. MINERALS: ASBESTOS, GLASS FIBERS, AND MICA

Asbestos and glass fibers can easily be identified and separated from all other fibers. Both are impervious to stains, but asbestos fibers decompose by fusion with sodium carbonate, and glass fibers melt in a flame. Mica is translucent and hard, with thin colorless flakes which cannot be broken with a needle.

GROUP XIX. PITCH

This group includes pitch, tar, and wood resin. The pitch specks, as a rule, are hard, friable and powdery, blackish dark-brown and shiny, but may be soft. They may show a slightly extensive halo and soften when treated with organic solvents. The tar specks are soft, spreadable and tacky. Pitch or resin from wood pulp has a yellow to reddish-brown color or may be practically colorless, and gives a red color reaction with the Raspail test.

GROUP XX. PLASTICS AND SYNTHETIC FIBERS

This group includes cellophane, plastics and synthetic fibers. Cellophane is translucent, soft, not spreadable, and usually regular in outline. Heated gently in a flame it does not melt, but burns and may evolve acrid vapors. Synthetic fibers are more or less opaque.

Specks from plastics are opaque, soft, not spreadable, more or less elastic and flexible. Heated in a flame they react similar to cellophane.

GROUP XXI. RUBBER SPECKS

Rubber specks are of two kinds, those which are soft, not spreadable, more or less elastic or flexible, and those which are soft, spreadable, and tacky. Both types, when heated in a flame may melt, giving an odor of burned rubber, and burning easily. Both are also slightly soluble in benzene.

GROUP XXII. SEEDS AND SEED HAIRS

Seeds and seed hairs may be air-borne or come from the raw material used for pulping. Cotton seed hull fragments, with the beard fibers attached to the narrow end of the seed, can easily be recognized by their brown or brown-black color.

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MICROSECTIONING

There are occasions when the microscopist wishes to examine the internal structure of paper or paperboard, or to study fibrous raw materials in greater detail. Frequently, these needs may be met by sectioning the object.

Materials which are relatively soft, firm, and tough, may sometimes be cut without further preparation by making free-hand sections, using a razor, or hand microtome. However, if sections of exact thickness are desired, a mechanical microtome is required, and it is often necessary to give the specimen special treatment before sectioning.

Special embedding methods must be used if the material is friable, of poor tenacity, or varying toughness, if the structures are loosely banded, or if very thin sections are required. The principal embedding methods are those involving paraffin, collodion, gelatin, and synthetic resins. The method used depends upon the purpose of sectioning as well as the nature of the object, and should not alter the structure or composition of the object or its chemical constituents.

THE MICROTOME

HAND MICROTOME

Razors such as those used for free-hand sectioning are commonly used with hand microtomes. The object is clamped in a holder and the knife is moved along a fixed course. In some devices the thickness of the section is fixed but in others a screw movement permits the preparation of sections of varying thickness.

Cross-sectioning devices like those described by Wöllhaf (1), Preston (2), Hardy (3), Schwarz (4), Herzog (5), Mennerich (6), Royer, et al. (7), Ford and Simmens (8), and Mannering (9), mentioned later under the sectioning of fibers, may also be considered as hand microtomes.

Hardy Microtome* (3)

This device comprises essentially two separate plates, A and B, which are frictionally held in proper relation by slide joint C (Figure 126). Plate B has a milled, smooth-walled fiber-receiving slot D, 0.0085-inch wide and 0.375-inch long. The companion plate carries a tongue which enters the slot D longitudinally, and fits into it within very close limits. The tongue is supported by a web which lies beneath the plate. Mounted on Plate B is a swiveling bracket E pivoting at F, and held in proper relation to the slot by means of a pin-lock G. The bracket carries a micrometer screw bearing a graduated head H readable by reference to an index I. The micrometer screw actuates an extruding plunger which may be made to enter the slot perpendicularly by turning the milled screw H.

*Manufactured by A. M. de la Rue, 3406 Longfellow Street, Hyattsville, Md. 20782.

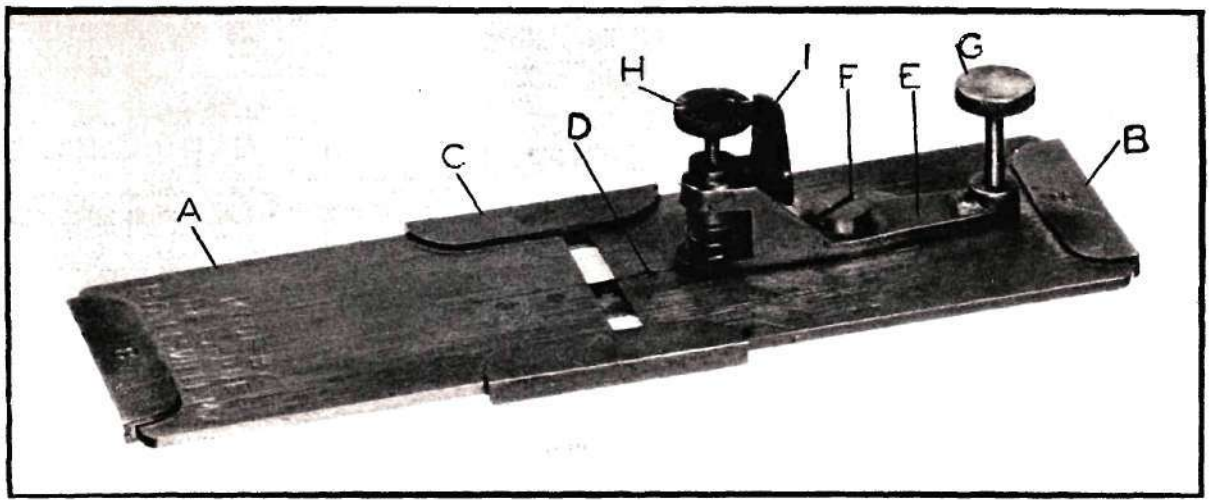

Figure 126. Hardy's Cross Section Device
(as Seen from the Underside)

In use, the two Plates A and B are separated, the lock G released, and the swivel E turned to a position transverse to Plate B. The fibers to be examined are packed into the slot D and compressed into a compact bundle by inserting the tongue and pushing the two Plates A and B into engagement. The excess fiber extending from either side of the plate is trimmed off flush by means of a knife or a safety razor blade. The bracket E is now turned to the position shown in Figure 126 and locked, the screw head H being turned until full abutment of the plunger with the bundle of fibers is obtained, as may be noted by a slight extrusion of the fibers observable with a glass on the reverse side of the plate.

MECHANICAL MIRCOTOME

There are several kinds of precision microtomes, but, in general, they may be classified as rotary microtomes, where the knife is stationary and the object is moved against the knife, the sliding microtome, where the knife moves in a guide against the object, and the clinical microtome, where the knife is pivoted at one end and moves against the object. In all types, the object is moved to the desired thickness for each cut.

In general, the rotary microtome is most suitable for ribbon cutting of serial sections from objects embedded in paraffin, while the sliding microtome serves for single sections or for sectioning with freezing attachments. The clinical microtome has not been useful in the sectioning of paper, fibers, or woody materials, but is valuable in pathological studies.

Several suppliers, here and abroad, manufacture various models of the different types of microtomes and most of them publish pamphlets on the effective use and proper care of the microtome [e.g., see (10) and (11)]. As in the purchase of all equipment, the reputation of the manufacturer as well as service facilities should be given careful consideration.

A good microtome knife must have a perfectly sharp cutting edge and must have the necessary stability both in the body as well as in the cutting edge. The latter requirement holds true especially for cutting hard and large objects.

The knives are either more or less hollow ground on one side with the other side perfectly flat, or have both sides plane ground. For easy-cutting collodion specimens, a plane-concave knife with the upper side considerably concave is selected; for harder collodion and easy-cutting paraffin sections, a less concave upper side is used; and for wood, hard paraffin objects, and frozen specimens, knives plane-ground on both sides are used (12). Knives of several lengths are available. For the sectioning of wood or paper the longer knives are preferred.

The microtome knife, like any other, is wedge-shaped and acts as such. The wedge form in microtome knives is changed, however, so that the cross section of the knife has five instead of three sides, since the cutting edge is formed by two narrow planes cut at a greater angle from the rest. These narrow planes make it easier to set the knife at the exact cutting angle.

The knife, when not in use, should be protected with a film of acid-free grease or oil before being returned to the knife case. When needed, it can be cleaned with xylene, rinsed in alcohol, and dried with a soft cloth.

SHARPENING THE KNIFE

The dulling of the knife usually involves both a loss of the cutting edge and the appearance of nicks. Resharpening usually includes a grinding of the edge to remove the nicks, followed by an extremely fine grinding and polishing to produce a new edge. In both hand and machine sharpening, the first grinding serves an additional purpose in that it fits the knife to the sharpening system so that when the fine abrasives or polishing materials are used they immediately act on the cutting edge. The condition of the knife edge should be assessed under the microscope during the sharpening procedure (13).

Hand Sharpening

A typical schedule of sharpening includes initial grinding on a yellow Belgian hone, finer grinding on a blue-green hone, stropping on a red rouge strop, and final stropping on a fine leather strop (14). In general, it is possible to sharpen a knife in about 15 minutes; much time is saved if the edge is not permitted to become too blunt.

During the sharpening procedure it is necessary to attach a device over the back of the knife to hold it in the correct position. A spring-back, split cylinder (Figure 127) is the usual attachment for this purpose, although other arrangements have been proposed (15). In addition, heavy knives are usually supplied with detachable handles to facilitate honing and stropping.

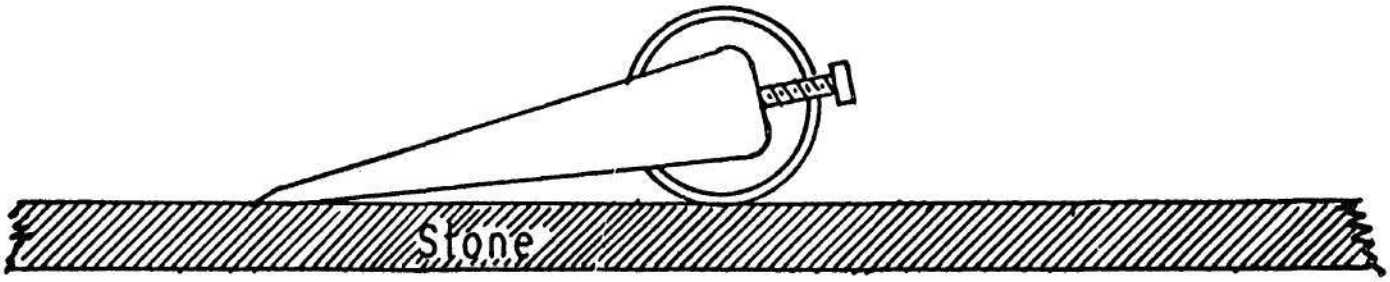

Figure 127. Correct Position of Knife on Stone

In sharpening, the knife with its backing piece is placed on the stone and pushed edge first from one side to the other; it is turned over on its back and the process is repeated. The entire edge should contact the stone during each stroke, as the tendency is for the middle section to receive more grinding than the ends, so that the edge becomes concave. If the blade is pushed along the stone obliquely, the whole edge is ground at each stroke and remains straight.

Stones should be cleaned of minute particles of metal and stone from time to time and should always be kept covered when not in use.

In stropping a knife, the blade is drawn along the strop back first. It is necessary to insure that both the back and the edge of the knife are kept in contact with the strop throughout each stroke, and at the end of a stroke the knife should be turned on its back, not on its edge. A solid strop should be used. The surface of the strop should be dressed occasionally with a strop dressing. Strops should be covered when not in use, as they pick up particles of dust and grit which damage the edge of the knife.

Sharpening may also be done on a piece of plate glass on which is spread a paste of very fine emery in water or olive oil. If a second plate, on which the abrasive is prepared chalk, is followed by a third over which rouge mixed with water is spread, subsequent stropping is unnecessary. Other schedules recommended use various grades of alumina or alundum and soap or detergent solutions (16).

Machine Sharpening

The tedium of maintaining the knife edge in a good cutting condition has led to the development of automatic sharpeners. In one type a large plate glass wheel rotates horizontally with the knife held at the proper angle and slowly swinging to and fro in a suspension of abrasive, such as levigated alumina (16). In another type, the knife is held manually at the correct angle in a trough and slowly passed back and forth against a vertically rotating precision plate glass wheel, 5/8-inch wide. An abrasive, levigated alumina, is used for sharpening, and liquid soap for the final polishing. Other devices, often homemade, have been described (17,18).

POSITION OF THE KNIFE

The position of the knife relative to the object is very important to obtain the best results. Two orientations must be considered: (a) Inclination, or the angle of tilt, which is the angle the knife makes with the horizontal plane of the object to be cut, and which is altered by turning the knife on its longitudinal axis; and (b) Declination, or the angle of slant, which is the angle the knife makes with the direction of the stroke (Fig. 128) (11,19d).

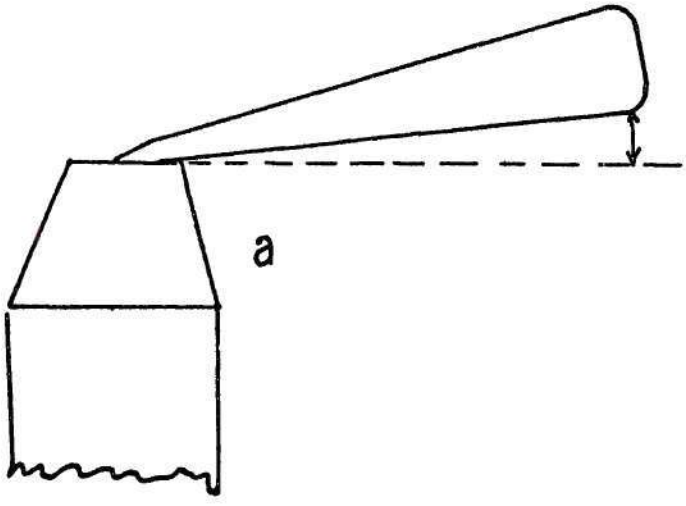

Figure 128a. The Angle of Tilt, or Inclination

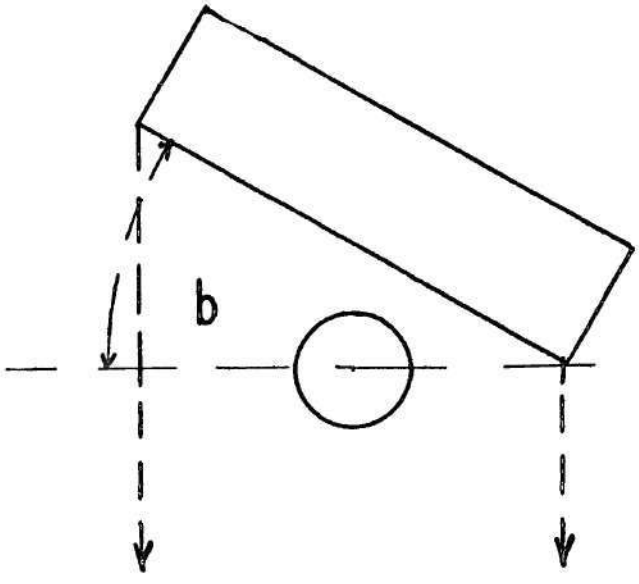

Figure 128b. The Angle of Slant, or Declination

General suggestions for the position of the knife:

(1) The knife blade and the surface of the object must form an acute angle; the softer the object to be cut, the smaller the angle. Kisser (20) suggests the inclination of the knife should be 6-10° for soft objects, 10-15° for harder objects, and more than 15° for very hard objects.

(2) The more acute the declination between the knife edge and the direction of the stroke, the less the cutting pressure upon the object, as the cut is made with a slantwise motion. The cut seemingly is sharper when larger portions of the knife edge are used. For soft objects, the angle is made as acute as possible; for harder objects, the angle can be somewhat larger; but even with very hard objects, the angle should not exceed 30-35°. Only in two cases can the angle be nearly 90° (perpendicular to direction of cut), when the object is embedded in paraffin, or frozen.

SELECTION OF MATERIAL

The material for microscopic examination should be chosen as representative of the timber, or otherwise suited for the purpose in view; this may best be done after inspection of a cleanly cut transverse section of the timber (21, 22). The specimen to be examined is cut into small blocks, so oriented that two faces are transverse, two are radial, and two are tangential. The block dimensions are about 3/8 by 3/8 by 5/8 inch (1 by 1 by 1.6 cm.), but it may be necessary to make the block smaller for the harder samples.

SOFTENING AND OTHER TREATMENTS

Blocks of wood that cut like hard rubber on the cross section with a sharp hand razor, should be ready to section with the microtome. The best sections come from blocks that are just a little softer than too hard. Freshly cut green wood, especially sapwood, can sometimes be sectioned without any special treatment; the surface of the block should be kept wet with water or alcohol. Seasoned wood generally requires a softening treatment to make it suitable for sectioning. The degree of softening required depends on the kind of wood and can best be decided by experience (21, 22).

The simplest method, suitable for fairly soft woods such as spruce and poplar, is to boil the blocks in water in order to expel the air which they contain; the process is considered complete when the wood no longer floats. The blocks will sink more quickly if they are removed from boiling water and washed in cold water, the two processes being repeated alternately until the block sinks.

An alternate method, which is to be preferred for fragile materials such as decayed wood, is to place the block in water in a vessel connected with a vacuum pump and exhaust the air. This is repeated several times until the block is thoroughly waterlogged.

Harlow (23) has listed a number of softening reagents, giving references as well as general remarks about the relative value of the methods. In all of these softening procedures, the objective is to prepare the specimen of wood so that it cuts transversely with the consistency of hard rubber.

Steaming Method

Good sections can be obtained from some unsoftened, dry wood samples by directing a jet of steam on the surface of the block as it is cut in the microtome. The method is quick and avoids the use of chemicals, but the condensation of steam on the microtome and the knife is objectionable (24-26).

Triethylene Glycol

In a method described by Burkart (95), 50-100 ml. of triethylene glycol are heated to 120-130°C. in a small beaker; the wood blocks are then immersed for 20-30 minutes, or until softened. It may be necessary to add 0.2-0.5% acid by weight (p-toluenesulfonic, phenolsulfonic, mineral, or Lewis acids have been suggested).

Glycerin and Alcohol

Some moderately hard woods are softened satisfactorily by soaking in a mixture of equal parts of glycerin and alcohol (denatured alcohol is suitable). After being waterlogged, the blocks should be transferred directly to the solution, and should remain immersed for about three days. This treatment is usually adequate even for fairly hard woods such as birch or oak (22).

When blocks intended for sectioning must be stored for any length of time, they may conveniently be given a slow softening treatment by immersion in glycerin and alcohol mixture containing not more than 10% glycerin.

Hydrofluoric Acid

Probably the method used most commonly to soften hard woods for sectioning is immersion in hydrofluoric acid (21, 23). For many timbers a mixture of equal parts of the commercial acid (60%) and water is satisfactory, the process requiring from about three days to a few weeks, according to the hardness of the wood. Very hard woods may require immersion in the undiluted acid for as long as six months. Specimens should be tested from time to time, after they are washed in running water for at least one hour. When the blocks are soft enough to cut, they must be thoroughly washed to remove the acid (a day in running water will usually suffice). Although the holocellulose of the wood appears to be partially hydrolyzed (27-29), the sections are quite suitable for anatomical study.

Hydrofluoric acid must be handled with considerable caution, as it is strongly corrosive and the fumes are toxic. It is best to wear rubber gloves when handling the acid. Since the acid etches and weakens glassware it must be stored in hard rubber, wax, or lead containers.

Peroxide and Acetic Acid

A satisfactory method for general use is that developed by Franklin (30, 31). A block of suitable dimensions is placed in a mixture of one part by volume of glacial acetic acid and two parts by volume of hydrogen peroxide ("20 volumes") in a glass flask fitted with a reflux condenser and is heated one to three hours, depending upon the wood species. The block is then washed in running water and is ready for sectioning. The method is very useful for purely anatomical studies and permits sectioning rapidly of even the hardest woods (22).

Embedding

To prepare badly decayed wood, charcoal, or otherwise embrittled wood for sectioning, it is usually necessary to impregnate the blocks with a material to support the fragile cellular structure. Collodion* has commonly been used for this purpose (32), but more recently it has been found that the methacrylates serve quite nicely (33) and also mixtures such as polyvinyl lactophenol (34). Wilcox (35), who investigated the celloidin, paraffin, and polyethylene glycol

*The highly purified substance used for embedding is known as celloidin, or Parlodion.

embedding methods, states that the celloidin method proved to be the most satisfactory procedure for embedding decayed wood. Modifications of procedures described later in this chapter are satisfactory, if one allows for the moisture content of the sample and proceeds accordingly.

SECTIONING

To cut cross sections, the block is clamped in the microtome with the growth rings parallel to the motion of the knife. Longitudinal sections are cut with the tracheids or fibers parallel to the motion. Tangential sections should be cut a little off the plane of the growth ring and through the border of a growth ring so that there will be both early and late wood on the section (21). Some workers advocate cutting the softwoods so that the knife meets the early wood first, and in hardwoods, the late wood first (22). Any preliminary trimming of the block surface should be done with the heel of the knife and the remainder of the edge reserved solely for sectioning. The knife is adjusted with an inclination of 10° with the surface of the block in the vertical plane and as acutely as possible with the line of motion.

To begin the sectioning, the knife, flooded with alcohol, is pulled in its slide with a steady, firm movement of the right hand, while a brush held in the left hand is placed gently on the block to prevent curling of the section; no attempt should be made to push the section onto the knife as this may cause tearing. By means of the brush, the section is then carefully transferred to a dish of alcohol. (With tangential sections it may be necessary to use a flooded spatula for this transfer.) Each time the knife is moved forward over the block a section is cut, and on the return stroke the block is raised the desired amount (10 to 20 μm.) for the next cut. The strong tendency to curl, shown by sections of some woods, can often be overcome by wetting the knife with a mixture of equal parts of glycerin and alcohol, and allowing each section to remain on the blade for a few seconds after cutting; the sections are then transferred to a dish containing the same mixture (22). Lee, however, recommends immediate transfer of the section to a Petri dish with enough 70% alcohol to cover it completely, followed by transfer to another dish with just enough alcohol to wet the sections, and allowing to stand overnight before proceeding (36). Another possible way to combat excessive curl is to leave several successive sections attached at one corner of the block (21).

An interesting method has been proposed which involves the use of cellulose tape, which is placed on the surface of the block before each section is cut. Sections as thin as 6 to 8 μm. were cut easily from the wood. The sections were remarkably flat, showed little or no rupture and no appreciable compression, and could be maintained in series on the tape (37).

It is possible to section wood freehand with a sharp razor or knife, but skill is required to prepare satisfactory sections, and even then the thickness of the section is frequently too great for many purposes. Some intriguing suggestions have been made for preparing wood sections; one of these requires only the use of an ordinary carpenter's plane and a softwood block, in which a hole may be drilled to insert the specimen (38); another uses dry samples sawed to 2-mm. thickness and impregnated with a resin, after which one side of the specimen is ground manually on abrasive papers with particles of increasing fineness (39).

STAINING

Although staining is not ordinarily recommended for woods with pronounced natural color, it is not unusual to stain the wood sections to provide contrast for anatomical study. A 1% aqueous solution of safranin is a satisfactory stain for woody tissues. The sections are moved from the alcohol solution in which they were placed after cutting to the safranin solution for up to 10 minutes. Then, they are gradually dehydrated by transferring through a series of solutions of increasing alcoholic content to absolute alcohol, before clearing and mounting.

Combinations of stains have frequently been used to stain wood sections; two commonly used procedures are given here, but many others are possible (12, 20, 40-42).

Safranin and Fast Green (22)

1. Cover the sections with 1% aqueous safranin stain for five minutes.

2. Drain the safranin stain and wash with at least three changes of distilled water.

3. Wash twice with 95% alcohol.

4. Cover section with 1% Fast Green in clove oil and alcohol (1:3) for five minutes.

5. Drain off the Fast Green and wash with at least three changes of 95% alcohol (one or two changes of absolute alcohol will suffice).

6. Transfer the sections to clove oil for five minutes, then to cedar oil for one minute, and mount in Canada balsam. If absolute alcohol is used in Step 5, xylene may be safely used in place of the cedar oil.

This stain combination differentiates lignified (red-staining) and unlignified (green-staining) structures.

Hematoxylin and Safranin (42)

Hexatoxylin may be applied to the sections with a schedule which requires previous treatment with an iron mordant, such as Heidenhain's. If the mordanting treatment is used, the wood sections are transferred from distilled water to a 4% solution of iron alum (ferric ammonium sulfate) for 30 minutes. After careful washing, the sections are immersed in 0.5% aqueous solution of hematoxylin for a few minutes and observed under the microscope for proper coloration. A drop of ammonia water will intensify the blue color. Be careful not to overstain, but destain if necessary; dilute mordant can be used for destaining. Wash the sections and stain in 1% aqueous solution of safranin for 5 minutes or more, if required. Then proceed to dehydrate in a series with increasing alcoholic content until the absolute alcohol stage is reached. If it is desired to stain with an alcoholic solution of safranin, this is used at the proper point in the dehydration series.

Hematoxylin may also be applied as a self-mordanting stain, of which the most important are Delafield's, Harris', and Mayer's. If the self-mordanting stain is used, transfer the sections into the dilute solution of hematoxylin for 5-30 minutes.

If overstained, the sections may be destained with dilute acid (1-5% acetic, 0.5% hydrochloric, or saturated aqueous picric). There are a wide variety of formulas available for preparing the stain (40-44).

MOUNTING

Canada Balsam Mounts

Transverse, tangential, and radial sections are transferred from cedar oil or xylene and arranged in the center of a clean microscope slide. Excess xylene can be removed by lightly touching with a blotter. Two or three drops of Canada balsam dissolved in xylene and of a molasseslike consistency are then placed on the sections, and a warm cover glass (25-mm. square) carefully lowered on them. The prepared slides are allowed to stand a day or so until the balsam gets tacky, and a spring clothespin is then applied to squeeze out surplus mountant. If clips are put on at once, too much balsam is squeezed out and air bubbles may be sucked under the cover glass as the balsam dries. The slides are kept in an oven at 60°C. for 12 hours and then allowed to cool. When hard, the surplus balsam is carefully scraped away and the edge of the cover slip brushed with xylene. The slide is finally polished with alcohol and labeled (21, 22).

A number of resinous mounting media, both natural and synthetic, have been proposed to replace Canada balsam for permanent slides. Some of these, such as euparal and diaphane, may be used directly from 95% or absolute alcohol, without a clearing agent. Some resins harden more quickly than does Canada balsam. Refractive index, color, brittleness, requisite dehydration and clearing must be taken into consideration with such recommended media (45, 46).

Glycerin Jelly Mounts

Temporary mounts may be made in glycerin jelly. If the sections have not previously been stained, a little 1% gentian violet (or other aqueous liquid stains) may be added and thoroughly mixed with the warm molten jelly. Sections are placed on the slide, a few drops of molten jelly added, a warm cover slip placed gently over the sections, and the slide heated until bubbles appear; a spring clothespin is then applied until the jelly has cooled and set. Sections containing alcohol should be washed thoroughly in water before mounting. Such mounts can be made more or less permanent by sealing the cover glass with paraffin, gold size, or Brunswick black (22).

The mounting medium is made with 5 g. gelatin; 30 ml. water; 35 ml. glycerin; and 5 g. phenol (dissolved in 10 drops of water). Dissolve the gelatin in the water at 35°C.; then add the other ingredients. Filter while warm through fine silk or coarse filter paper. This mounting medium keeps well. When needed, a small amount is melted for use. Continual remelting causes deterioration (42).

SECTIONING OF PLYWOOD AND IMPROVED WOODS

Small specimens of plywood or veneers may be sectioned after being softened by refluxing in a solution of one part by volume of glacial acetic acid and two

parts by volume of hydrogen peroxide ("20 volumes") at 60°C. and atmospheric pressure (30). A 10% aqueous solution of sodium hydroxide for two hours with refluxing may be used to prepare "Compreg" for sectioning (47). These treatments have a softening action on fully cured phenol-formaldehyde and urea-formaldehyde resins and on wood-resin composites.

A modification of the acetic acid and peroxide treatment, using equal parts by volume at 60°C. for 48 hours, has the effect of decomposing or disintegrating a urea-formaldehyde resin. Phenol-formaldehyde resins are softened to an extent that "improved wood" incorporating such resins is broken down and the wood itself macerated.

Transverse and radial sections of rotary-cut veneers can be prepared by holding them in the microtome chuck between pieces of wood of similar hardness. To obtain tangential sections (parallel to the face) a strip of the veneer is bent around a small block of wood so that the material may be securely clamped in the microtome with a tangential surface uppermost (22).

SECTIONING OF FIBERS

A number of methods are available for preparing cross sections of fibers. Some are relatively rapid, using fairly simple techniques, whereas others require elaborate preparation and the use of a mechanical microtome. Choice of method depends on the type of fiber, the purpose of the section, whether it is for quick identification or a detailed examination, as well as the time available.

The type of embedding for dry fibers and fiber bundles is selected according to the relative swelling and area increase of the fiber cross section with the different embedding media, as well as the relative hardness. Since many other solvents besides water have a marked swelling action, it is obviously advisable to use considerable caution in the selection of embedding media, clearing agents, and mountants (48).

HAND TECHNIQUES

Plate Method (1, 2, 19a, 49-51)

A metal, glass, or plastic plate of the same dimensions as an ordinary microscope slide, contains a number of holes for holding the textile fibers. In general, five of the holes are 1/64 inch in diameter, and the other five are 1/32 inch in diameter. A bundle of fibers is drawn through a hole with a fine wire loop, or by a loop of fine thread, and cut evenly at each surface with a safety razor blade or knife.

After the fibers have been cut, the ends are covered with a drop of glycerin and a cover glass to reduce light scattering. The condenser should be adjusted to focus a small cone of light on the hole in the plate. Contrast can be increased by applying a smear of a black embedding medium, such as glycerin jelly and India ink, to the tuft of fibers, to fill the spaces between the fibers prior to cutting (52, 53).

Cork Method (2, 19a, 49-51, 54)

Another simple method which can be used with a greater variety of fibers is the cork method of Viviani (55) and Herzog (56). A threaded sewing machine needle is forced through the center of a small, good quality cork. After the loop is secured, the needle is withdrawn. As many fibers as possible are placed in the loop, and the fibers are carefully drawn through the cork by means of the loop. A sufficient number of fibers should be taken to ensure a slight compression, so that the fibers are held firmly in position without being distorted.

The surface of the cork is cut perpendicular to the direction of the fiber bundle. A moderately thin slice, about 0.5 mm., is cut from the cork with a sharp razor blade. When cut, the section (cork and fibers) is placed in a drop of water or glycerin on a cover glass. The cover glass is turned over and placed upon a perforated slide or a ring cemented to the slide. The section remains glued against the cover glass, so that the cutting level is homogenized. When the cork has been levelled off, however, it may be preferable to brush a thin coating of collodion over the cork and then let it dry before the cross section is cut.

For the sectioning of very stiff, thick fibers, a cork is split lengthwise into two equal parts. In one of the halves a groove is made, in which the fiber bundle should fit snugly. Then place the halves together and, if necessary, tie with thread. If the fibers are too hard they must be soaked in equal parts of glycerin and alcohol for two days before being placed in the cork. Sections 30 μm. thick can be cut freehand or with a hand microtome (19a).

When only a few fibers are available, they may be placed in a cork which has been split lengthwise to about two-thirds of its thickness. The two parts are separated by bending and the fiber bundle placed in the slit; the elasticity of the cork will clamp the fibers sufficiently to hold them (57).

Hardy Microtome Method (3, 50, 51, 58)

The Hardy microtome described earlier in the chapter, or a modification of it devised by Schwarz (4) or Royer, et al. (7), is very useful for rapid fiber sectioning.

A drop of collodion is placed on the end of the bundle of fibers exposed opposite the plunger, allowed to dry, and the slightly projecting end is sliced off and discarded. The graduated micrometer screw is now turned enough to give the desired section thickness. The solution is again applied, allowed to dry, and a new section cut. This slice is suitable for mounting with Canada balsam, but other resinous or liquid mounts of a temporary nature may be used. Several additional sections may be made, if desired; the minimum thickness is about 25 μm.

RAPID METHODS FOR MECHANICAL SECTIONING

In the rapid embedding methods the fiber mass is only enveloped with the embedding material, consequently relatively little time is required to prepare cross sections. If a more homogeneous block is required for microtome sectioning,

one of the detailed methods should be used. In this way thinner and more perfect sections are usually obtained for examination (19b).

Paraffin Candle

This method is used chiefly to make cross sections of rayon fibers. The unstained fibers or threads are dipped or squeezed into black shoe polish, dried, treated again with the polish, and then embedded in paraffin just above its melting point. The embedding may be done in a frame or by repeated dipping to build up a candle. The black polish offers an excellent contrast for the light-colored fibers (59).

Collodion

This method is used for embedding wool and hair fiber and is also recommended for very hard fibers such as abaca and sisal (60).

Uncolored bundles of fibers are arranged parallel to one another on a dark, stiff paper in which notches are made at both ends to fasten the fibers. The paper is fastened upon a board with thumb tacks and the ends of the fibers securely fastened with collodion wax (one part wax and two parts collodion). Pieces of oil paper are placed over the treated portion to protect the ends from the embedding medium. A solution of collodion in acetone is then poured over the fibers, beginning in the middle to let the air escape; enough solution is poured over the fibers to form a solid mass. After drying for 2-6 hours, the single fiber bundles can be cut and placed between cork or small wood blocks in the microtome for sectioning. A color contrast may be obtained between the fibers and the embedding medium by adding Sudan III to the solution.

Collodion and Paraffin

This method may be used for very short fibers which cannot be handled with the plate or cork methods (61).

The short fibers are firmly pressed against two rayon threads which have been covered with collodion. When the fibers have been pressed into the thick solution, they and the rayon threads are again covered with collodion. After careful drying the whole is embedded in paraffin, either by placing in a frame or by dipping to form a candle.

If the short fibers are colored they are pressed against uncolored rayons, and vice versa. Rayons of known cross-sectional characteristics should be used.

Cross Sectioning of Pulp Fibers (IPC Collodion Method)

A 0.05-0.10% water suspension of pulp fibers is prepared. About 2 ml. of this suspension is spread over a part of a clean 5- by 7-inch glass plate. The suspension is tapped with a dissecting needle until the fibers are uniformly distributed. Then the point of the needle is placed against the plate and moved in a more or less curved line through the suspension, thus gathering a clump of fibers around the needle point. This fiber clump is transferred to a dry, clean slide and, by careful manipulation, the two ends of the clump are straightened. In this way, most of the fibers lie parallel to each other. In a like manner, more fiber clumps are gathered until a fiber bundle of the desired size is formed.

The bundle is treated successively with 95% alcohol, acetone, and amyl acetate by adding these solvents to the bundle with a fine-tipped dropper and allowing the excess to drain away into a small beaker, being careful not to disturb the orientation of the fibers during the process. While the fibers are still wet with amyl acetate, they are covered with a rather large drop of dilute collodion. Any disarranged fibers are straightened and small air bubbles are removed with the dissecting needle. The first portion of the embedding medium is allowed to partially dry, but before complete dryness a second drop, and subsequent drops of sufficient volume to build up a layer of the proper thickness for cutting are added. Any small air bubble appearing in the film is broken up or removed with the dissecting needle. If necessary, a small amount of amyl acetate is added to facilitate the removal of air bubbles.

The final film of the proper thickness is allowed to dry completely at room temperature, and is then stripped from the slide by means of a razor blade. The film is trimmed with a small, sharp scissors to a small size which includes only the fiber bundle. The small strip of film containing the fibers is then placed in the slot of the Hardy microtome with the fibers oriented at right angles to the flat surface of the metal plate. The excess of film on each side of the plate is trimmed off with a razor blade and the cross-section device is assembled in the proper position for cutting sections. The micrometer screw is advanced slowly and sections of the proper thickness are cut. The first few sections are discarded. A number of sections (6-8) are cut in the normal manner, and these are placed to one side on a clean microscope slide. The cut sections are mounted on the slide with Canada balsam under a cover glass after arranging the sections in two neat rows with a little dilute balsam and the help of two dissecting needles. The embedded fibers may also be mounted between two small strips of wood or cork for sectioning in the mechanical microtome.

Cross Sectioning of Pulp Fibers (IPC Methacrylate Method)

The fibers to be sectioned are stained with a dilute solution of Congo red and then brought into a suspension whose consistency is such that one drop contains about 50 fibers. A drop of the suspension is placed on a glass slide and all fibers aligned parallel to each other with the aid of dissecting needles and a Greenough-type binocular microscope. The clump of fibers is allowed to dry.

Place the fiber bundle in absolute alcohol for 20-30 minutes, then in a 50/50 mixture of absolute alcohol and butyl methacrylate for 20-30 minutes and finally in butyl methacrylate for 30 minutes. Transfer to butyl methacrylate plus 1% benzoyl peroxide catalyst briefly and then orient the fiber bundle in a gelatin capsule (No. 00) and fill the capsule with a fresh solution of resin plus catalyst; put on the top of the capsule* and place in an oven overnight at 45-50°C. The inhibitor is removed from the resin by treating with dilute caustic soda, washing and neutralizing, and carefully dehydrating. Fibers which are not properly dehydrated actually swell or are distorted upon polymerization of the methacrylate.

To minimize the tendency of wood fibers to collapse, particularly the thin-walled softwood springwood tracheids, freeze-drying has been used. A suspension

*A suitable polyethylene capsule is manufactured by BEEM (Better Equipment for Electron Microscopy Inc.), P. O. Box 132, Jerome Ave. Station, Bronx, N. Y. 10468.

of stained fibers is frozen and then transferred to a drying apparatus. A sample of the freeze-dried fibers is then placed in a drop of butyl methacrylate on a glass slide and aligned parallel to one another, as described previously. The saturated fiber bundle is carefully removed from the butyl methacrylate with a dissecting needle and placed in a gelatin capsule previously filled with butyl methacrylate and catalyst for final embedding.

DETAILED EMBEDDING FOR MECHANICAL SECTIONING

Paraffin Embedding Method (11, 19c, 42, 49, 54)

Dehydration

The specimen is dehydrated, if necessary, by soaking in a series of water-alcohol solutions of gradually increasing strengths up to absolute alcohol. If the material is hard or dry and brittle, it is soaked in a mixture of equal parts of alcohol and glycerin until soft.

Clearing

The alcohol is replaced by soaking (24 hours or less) in a liquid which is miscible with paraffin, such as xylene, benzene, cedar oil, clove oil, or chloroform. This is commonly done in successive steps of increasing strength in the clearing agent.

Fusion with Paraffin

The fusion is carried out by one of two methods: (1) The solvent-saturated object is placed in melted paraffin (just at its melting point) and kept there until thoroughly saturated. (2) The solvent-saturated object is put into an open vessel (on a cold water bath) containing a saturated solution of paraffin and solvent (xylene is the common solvent used). The bath is gradually warmed to 60°C. in two hours and, as rapidly as the solvent evaporates, melted paraffin is added to the vessel. The paraffin should be protected from water vapor while on the water bath. When the object has been thoroughly impregnated with paraffin, it is allowed to stand in an open vessel until the solvent has evaporated.

Embedding

The paraffin-saturated object is embedded in a mold by one of the following methods.

A tray is made from paper, some melted paraffin is poured into it, the object is placed in the middle of it, and fastened in the correct position with needles (41, 42). Multiple molds have been suggested for paraffin embedding, one of silicone rubber being most adaptable (62).

If it is desired to fix the object at a definite position, very small objects may be oriented as follows: The object is removed from the melted paraffin and placed in a cylinder of solid paraffin; a needle or stout iron wire is heated in a flame and used to melt a hole in the end of the cylinder; the specimen is then pushed into the melted paraffin, and placed in any desired position. In using the

needle it is important to melt as little paraffin as possible at one time so that the melted portion may cool quickly. This method is rapid and positive.

Another method of embedding is to dip a small bundle of the fibers in melted paraffin, just above the melting point, and then remove it quickly, place in ice water, dry with soft cloth or paper, and repeat several times until a sufficient thickness of paraffin adheres to the fibers.

The properties of the embedding paraffin are important factors in the success or failure of sectioning (42). The melting point of the paraffin should be selected according to the temperature of the laboratory. For thin sections a harder paraffin is required than for thick ones. Hard objects require harder paraffin than do soft ones. Old paraffin, which has been melted many times, is better than fresh, because it has less tendency to crystallize.

Preston (2) claims that paraffin alone is not sufficiently hard for sectioning cotton, bast fibers, and silks and recommends a mixture of equal parts of paraffin and stearic acid; Osborne (63) suggests that 0.5% bayberry wax should be added to the paraffin; Barlow (64) proposes different mixtures of stearic acid, ethyl cellulose, and paraffin wax; Steedman (65) prefers a mixture of diethylglycol distearate, ethyl cellulose, stearin, and ricinoleic diacetate; and Walter (66) uses a mixture of carnauba wax, candelilla wax, beeswax, rosin, and Venetian turpentine.

Cooling

Whichever method of embedding and orientation is used, it is necessary that the paraffin be cooled as rapidly as possible to prevent crystallization and to obtain a homogeneous mass. As soon as the paraffin is hardened enough to keep the object from moving, float the tray in a pan of ice water. Allow the surface to solidify and submerge in water until the mass is thoroughly cooled. White areas or bubbles in the wax can be minimized or prevented by storing the blocks in a refrigerator for several days.

Cutting the Sections (19d)

The paraffin blocks or candles, after being trimmed, should be cemented with melted paraffin between small blocks of wood or some other solid material and fitted into the chuck of the sliding microtome; the paraffin block is too weak to be clamped directly in the object holder.

The rotary microtome with a fixed knife will give good results with harder paraffin. The paraffin block is cemented onto the metal mounting disk with melted paraffin.

If a ribbon cutting is to be made, the first section remains on the knife, the following adheres to the first one, and so on until the desired ribbon length is formed. The temperature at cutting is significant because if it is too low or the paraffin too hard, the sections will roll up on themselves on the knife. This also occurs if the sections are too thick. On the other hand, if the paraffin is too soft or the knife too blunt, the sections telescope and become useless. This can be corrected by lowering the room temperature.

If ribbon sectioning is not desired, better individual sections are obtained with a slanting knife, although sections cut with a slanting knife sometimes curl.

This can be overcome by changing the speed of the stroke because with more rapid strokes there is less chance for curling. It has been suggested that transparent adhesive tape be used in the preparation of microsections of paraffin-embedded textile fibers (67).

Under ideal conditions the thinnest sections which can be made in paraffin are 2 μm. thick, but, in general, fiber cross sections should not be cut too thin.

Fixation of Section to Slide

Short sections of the ribbon or individual sections are fastened to the slide with Mayer's albumin fixative. A very small drop of this is placed on the slide, smeared over the entire slide with the ball of the finger, and then rubbed thoroughly with a piece of chamois so that only a very thin layer remains. Several drops of distilled water are placed on the slide and the sections are superimposed in the desired position. The shiny side of the section is placed in contact with the water. The sections will then stretch themselves and this effect can be increased by carefully heating the slide in such a manner that the paraffin does not melt. Remove the excess water with filter paper and dry the slide in a warm dust-free place.

Removal of Embedding Medium

The slides are than placed upright in a staining dish (Coplin jar) filled with xylene, and when the paraffin has dissolved, the slides are quickly transferred to a clean xylene bath.

Staining and Mounting

The sections can then be mounted directly in Canada balsam or other mounting medium. The choice of the mounting medium is of great importance. It may be a liquid or resinous substance, and there must be sufficient difference between the refractive index of the medium and that of the object so that the object can be seen to its best advantage. A rather complete list of mounting media and their refractive indices may be found in Chapter II (Table II). If it is necessary to use a mounting medium with a refractive index near that of the object, the object must be stained.

If the sections are to be stained, the slides are transferred to successive mixtures of equal parts of xylene and alcohol, 85, 70, and 50% alcohols, etc., depending upon the alcoholic content of the stain to be used. After staining, the sections are again dehydrated and cleared with the solvent used for the mounting medium.

Glycerin-gelatin mounts are not as permanent as those in resin or balsam, but can be kept for several years, if properly sealed.

The mounting medium is prepared as follows: 300 g. of the best dry gelatin are soaked for two hours in 1000 ml. of water heated to 50°C., 10 ml. of phenol at 50°C. and 500 ml. glycerin are added, and the temperature is held at 50°C. until the phenol completely dissolves. The solution is filtered through double filter paper on a hot water funnel into a dish where it is allowed to cool and solidify. Small pieces or blocks are cut and placed on the slide which is carefully heated to 60°C., without boiling the solution. The object which has been

kept a short time in a glycerin bath is then transferred to the melted glycerin-gelatin medium, covered with a heated cover glass, and the excess liquid removed with filter paper.

Collodion Embedding Method (2, 11, 19c, 20, 40-44, 49, 54, 58, 68)

Collodion* masses do not require the use of heat for embedding. They are more or less transparent and remain quite transparent after mounting; hence, the matrix need not be removed before staining and may be used as a support for holding brittle or detached elements in place, which otherwise would fall apart and be lost. The embedding process is quite long, however, requiring three days for objects that can be embedded in an hour in paraffin. The use of pressure vessels during the procedure can reduce the time somewhat. Sections cannot be cut as thin when embedded in collodion as in paraffin.

Dehydration

The object is very thoroughly dehydrated in a series of solutions of increasing concentrations of alcohol into absolute alcohol. It is then soaked in an equal mixture of alcohol and ether for 24 hours. It is placed next in thin collodion (4-6%) solution for 24 hours to obtain complete infiltration of the embedding medium, and finally transferred to a thicker (10-12%) solution for 24 hours.

Embedding

The embedding vessel, which may be a small paper or cellophane cup or other container, should be prepared for the reception of the object by first pouring a small drop of collodion (sufficient to cover the bottom) into it and allowing it to dry. This is done to prevent air bubbles arising from the receptacle when the rest of the collodion is poured. A portion of the thick solution of collodion is then poured into the receptacle and bubbles removed by exposing the solution to ether vapors in a desiccator for a few hours. The specimen previously impregnated with collodion is placed on top of the thick collodion in the receptacle and the mass set under a glass beaker or other container, so arranged to allow evaporation. As soon as the solution has evaporated so that the object begins to lie flat, more of the solution is added, and the evaporation continued. If the first layer has become dry, it is moistened with a drop of ether before adding more of the solution.

Hardening

When the collodion mass has such a consistency that the ball of the finger does not leave an impression, it is placed in chloroform. In some cases a few hours are sufficient to harden the collodion and not more than three days should be needed. Another common hardening method involves the use of alcohol, in which from one day to several weeks may be necessary. From the viewpoint of cutting and transparency, 85% alcohol is the best bath. Hardening in this grade of alcohol, the mass must be kept moist while cutting, since it dries through evaporation. The hardening blocks of collodion may be preserved in 70% alcohol and should be kept dry by dipping in paraffin or, after rinsing with water, should be placed in glycerin jelly, which is removed with warm water before cutting.

*Collodion, or pyroxylin, are lower forms of cellulose nitrates. The highly purified substance used for embedding is known as celloidin, or Parlodion.

Another suggested procedure follows: The object is dehydrated, soaked in ether, and put into a test tube of collodion solution. The tube is dipped into a bath of melted paraffin, and the collodion solvent is allowed to boil (this occurs at a very low temperature) until the collodion has a sirupy consistency; the final solution should be about one third of its original volume. The mass is removed from the tube, mounted on a block of hardened collodion, and hardened in chloroform or in a mixture of chloroform and cedar oil for about one hour. It may now be fixed in a microtome and cut, using cedar oil to wet the knife. The exposed surface of the object is covered after each cut with a thin layer of collodion. Small objects may be prepared in an hour, compared to days required by the older methods.

Sectioning

To fix the collodion block in the microtome, a piece of soft wood or cork of the size and shape adapted to the object holder is covered with a layer of collodion, and allowed to dry. The surface is wet with absolute alcohol and then with ether; one drop of very thick collodion solution is put on the prepared wood or cork, and the block pressed down firmly; this is now placed in 70% alcohol for a few hours or, preferably, in chloroform for a few minutes to harden the joint.

Both knife and specimen should be kept wet with alcohol (50-95%), and the knife should be as oblique as possible to the specimen and moved with a draw stroke which is quick and steady. It may be helpful to paint the exposed surface of the object after each cut with a thin layer of dilute collodion and allow it to dry before making the next cut.

Mounting

The collodion sections, which are temporarily placed in 70% alcohol immediately after sectioning are transferred to a clean slide and the alcohol is removed with filter paper. The slide is transferred to a covered dish which contains a small basin of ether. Under the influence of the ether vapor the collodion softens and adheres to the slide when removed from the dish. It is unnecessary to remove the collodion from the sections.

Another method involves spreading a very thin layer of collodion on the slide before the sections are placed on the slide and exposed to the ether vapor, or fastening the sections on a thin layer of gelatin and hardening the whole with formalin.

In still another method the section is fixed to a slide with a small drop of ether. The section is flooded in 95% alcohol for 30 seconds and then with absolute alcohol which contains about 5% chloroform to prevent the alcohol from dissolving the matrix. Clearing may be effected with xylene, or better with benzene. A few drops of Canada balsam, preferably dissolved in benzene and somewhat thicker than that used for paraffin sections, is placed on the sections, and a cover glass added.

The matrix may be removed, if desired, between the last absolute alcohol and clearing agent stages, with ether or a 50-50 alcohol-ether solution. If it is desired to stain the fiber sections this may be done at the appropriate alcoholic concentration, with subsequent dehydration.

Plasticized Collodion

Lee and Schwarz (69) recommend the following formula for a plasticized collodion to be used as an embedding medium:

23.0% dehydrated nitrocellulose (5-7 seconds cotton); 23.0% No. 1 castor oil; 0.8% mineral oil; 24.5% ethyl acetate (90%); 9.0% toluene; 18.2% naphtha, low flash; and 4.5% butyl acetate.

Double Embedding in Collodion and Paraffin

This procedure is used with materials that combine hard tissues with regions of very fragile and brittle tissues. Embed the object in collodion and harden well in chloroform. The surrounding collodion is trimmed, exposing all cut surfaces. The chloroform is changed several times to eliminate the collodion solvent. The mass is then embedded in paraffin. It may be possible to section on a rotary microtome and obtain a ribbon, but in some cases it will be necessary to use a sliding microtome. The sections are fastened to the slide in the same manner as paraffin sections (41, 42).

Gelatin Embedding

This method differs from others in that the objects are prepared by saturating with water instead of alcohol or a clearing agent.

Twenty grams of gelatin are dissolved with heat in 200 ml. of distilled water, the solution filtered, and 30-40 ml. of glacial acetic acid and 1 g. of corrosive sublimate added. The object, which is water-saturated, is put into the above solution diluted with two to three volumes of water, and then transferred to the undiluted solution. The mass is hardened in a solution of potassium dichromate, picric acid, alcohol, and formaldehyde, or it may be frozen.

Sectioning

The gelatin blocks, when properly hardened, can be placed directly between two pieces of wood and placed in the microtome chuck. In many cases, the hardened mass can be placed directly between the jaws. The knife should be slanted as much as possible and sections can be cut as thin as 10 μm. The knife should be kept wet with alcohol during the cutting operation. The sections are placed in 70% alcohol and then transferred to an albumin-glycerin-treated slide. The alcohol is removed with filter paper and the sections are fastened by heating the slide to 65°C. The gelatin may be removed with warm water or can be dissolved in a 5% solution of calcium chloride in 70% alcohol.

To embed certain fibers (e.g., dyed cellulose acetate), where it is necessary to avoid the use of organic solvents such as alcohol or chloroform, Lawrie (68) recommends gum arabic as an embedding medium. This method is also used by Lee and Schwarz (69) and Preston (2).

Synthetic Resin Embedding

In recent years several procedures involving the use of various synthetic resins have been tried for embedding fibers. The methacrylate resins, especially methyl and butyl, and the epoxy resins, such as Epon 812, are among those most

frequently used. The epoxy resins appear to result in considerably less shrinkage, as little as 1-2%, and so may be preferred by many users.

A suggested method (70) for fibers, using Epon 812, follows, but new procedures are being recommended for ultrathin microtomy and it may be feasible to adapt some of these suggestions.

If the fibers are moist, they are treated successively with 50% alcohol for 10 minutes, 70% alcohol for 10 minutes, 95% alcohol for 10 minutes, and absolute alcohol for 15 minutes, all at room temperature. If the fibers are dry, the treatment may begin with absolute alcohol. It is important that dehydration is complete.

The subsequent steps are carried out at 40°C. in an oven. The fibers are placed in an equal mixture of absolute alcohol and Epon 812 for 15 minutes, then Epon 812 for 30 minutes, then two changes of 1-2 hours each in a mixture of Epon 812 (10 ml.) and DDSA hardener (19 ml.) (dodecenyl succinic anhydride), and then in two changes of 1-2 hours each of this mixture plus 1% BDMA accelerator (benzyldimethylamine)*. If this time is appreciably extended, it is difficult to free the fibers and resin of trapped air bubbles after transfer to the gelatin embedding capsules (No. 00) for final hardening overnight at 40°C. before sectioning with the sliding microtome. In some cases, with fibers, it has been possible to shorten the time with the resin and hardener, and the mixture plus accelerator.

For sectioning, the harder epoxy mixtures are sufficiently rigid to resist plastic deformation in the microtome chuck, and there is consequently no need to cement specimens to a dowel or block. The sections are treated in a manner similar to the collodion-embedded sections.

SECTIONING OF PAPER

CROSS SECTIONING OF PAPER

Methods to be used for the sectioning of paper depend upon the purpose of the study, such as the relative thickness of coating, penetration of ink, thickness of different layers and binding materials, distribution of fillers or sizing, and the examination of felting characteristics or minute surface structures of a paper or board. For the same reasons, the relative thickness of the cross sections and the embedding medium, as well as the type of illumination used for the microscopic examination, is of utmost importance.

For extremely thin sections in which individual fiber shrinkage and paper expansion may be disregarded, the paraffin or resin embedding methods may be the best. If somewhat thicker sections are permissible, a combination cork-paraffin method is useful, and for rough cross sections, where little time is available, a plain cork method can be used; however, if fiber shrinkage and paper expansion

*Hardener and accelerator obtainable from R. P. Cargille Laboratories, Inc., 117 Liberty Street, New York, N. Y. 10006.

must be avoided, or if dehydration, clearing agents, solvents, or heat will destroy the character of the paper to be sectioned, a collodion-cork method is recommended. No matter which method is used in making any cross section, it is necessary to have the microtome knife as sharp as possible for good results.

In mounting paper sections it is important that the dehydration operation or clearing agent does not remove, destroy, or change any of the substances in the paper or paperboard.

Freehand Methods

The simplest and most readily prepared type is the freehand section. There are two types of freehand sections. In the first type a wedge of the sample is placed on a smooth, solid surface and a sharp straight cut made with a freehand sectioner, which consists of a razor blade mounted in a holder. If a specific area is to be sectioned, it is best done under a low power stereoscopic microscope (10X). The sliced sample is trimmed to a convenient size and placed in a clamp with the cut edge up. The clamp should be so devised that it may fit into the mechanical stage of a microscope. The section must be viewed by reflected light. Stains and reagents may be applied to the cut edge for such purposes as to delineate the interface between two layers. These sections may be prepared in a few minutes (71).

A second type of freehand section produces a thin slice of the sample. After making the initial cut with a freehand sectioner, as described above, a second cut is made beside the first to obtain a thin section. With a little practice, sections 30-40 μm. can be cut. These sections can then be mounted on a microscope slide and examined by either reflected or transmitted light. This thin type section can be prepared in a few minutes (71, 72).

Hardy Microtome

Cross sections of paper and film can be cut satisfactorily with the Hardy hand microtome. Strips of paper, 4.5-mm. wide, are placed in the slot of the instrument and the space left vacant is filled with fibrous material, such as coarse wool, mercerized cotton, etc. The purpose of this packing is to hold the specimen firmly in place. Surplus paper and material are cut off, and the surface is coated with collodion, which is allowed to dry hard for 20 minutes. The section is then prepared by a cutting action of the blade and placed, cut surface upward, on a microscope slide, and mounted in Canada balsam or other suitable medium. The paper can be stained either before or after sectioning; films are treated in exactly the same manner. After some practice, a complete section can be prepared in about 10 minutes (73).

Jung Hand Microtome

A simple small cylinder-shaped hand microtome, with a pincerlike object holder, manufactured by Richard Jung Company of Heidelberg, Germany, permits cross sections of papers and films to be made without pretreatment. Under some conditions, sections as thin as 20 μm. may be cut, but generally the sections obtained are about 30 μm. thick. Best results are obtained with coated papers, laminated papers, reinforced papers, etc. Loose-structured papers do not give as good results as high density materials, even though extremely sharp knives or new razor blades are used (74).

Sandwich Methods

One of the earliest techniques used for sectioning paper was described by investigators at the Eastman Kodak Company in 1917 (75, 76). A small strip of paper was mounted between two pieces of ordinary photographic film, the gelatin coating of which was moistened to cause it to adhere to the paper. Film was used because the gelatin coating acts as a firm, though slightly resilient, binder and prevents the tearing of the surface fibers. The paper and the film were next placed between two pieces of moderately dry castile soap, which was placed in the chuck of the sliding microtome with the paper edge perpendicular to the knife edge. Sections were cut about 30 μm. in thickness, mounted in Canada balsam on standard microscope slides, and examined.

Graff and Schlosser (77) used a variation of this technique and were able to prepare sections about 15 μm. in thickness. They mounted the paper strip between two ribbons of nonmoistureproof cellophane in a slit in prepared cork (see next section). By means of a pointed glass rod, a tiny drop of sodium silicate is placed at each end of the paper strip to prevent the separation of the section from the cork during the cutting operation. After the sodium silicate has dried, the section is cut. The cork and cellophane may be removed before the section is mounted.

Investigators at the Forest Products Laboratory use a sandwich technique with cellulose acetate film which is softened with acetone before the paper specimen is placed between them and pressed (72).

Cork Method (77)

A strip, 4-5 mm. wide and 5 cm. long, is cut from the paper sample. It is dehydrated in 50% alcohol for ten minutes, transferred to 95% alcohol for ten minutes, cleared with xylene for ten minutes, and then transferred to a saturated solution of paraffin in xylene for 30 minutes or longer to impregnate the paper. Paraffin with a melting point of 56-58°C. is satisfactory.

New No. 1 corks are prepared by being weighted in the bottom of a vessel, covered with 50% alcohol, and heated to the boiling point of the alcohol for 30 minutes; the alcohol is decanted, the corks covered with 95% alcohol, and again boiled for 30 minutes; after pouring off the alcohol, the corks are covered with xylene and heated for 30 minutes; the xylene is discarded and the corks are heated almost to the boiling point with a saturated solution of paraffin and xylene for one hour. Finally the corks are placed on a paper towel to dry and then stored in wide-mouthed bottles. The total time needed to impregnate the corks with paraffin is about two and one-half hours.

With a razor blade a slit is made parallel to the long axis and at least half way through the cork. The strip of paraffin-impregnated paper is inserted longitudinally into the slit, and the cork and paper are immersed in a bath of melted paraffin for 30 minutes. A dissecting needle inserted in the cork will hold it beneath the surface of the bath. After the required length of time, the cork with its embedded paper is removed from the paraffin, cooled, and mounted in a microtome.

The cork is oriented in the chuck with the slit in the end parallel to the jaws. This prevents opening the slit because of pressure on both sides from the

object holder. The knife is set with a very small angle of slant (about 8°) and with a tilt of about 15°. The object holder is turned until the leading edge of the knife, acting as one side, and the paper as the other, make an angle of about 50° (Fig. 129). On a sliding microtome, sections of 30 μm. can be cut.

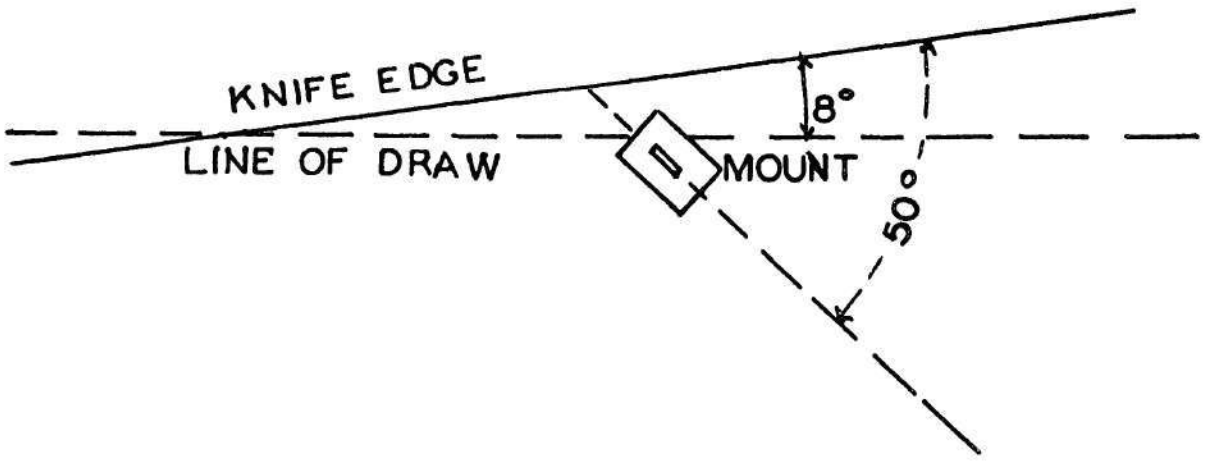

Figure 129. The Relative Position of the Knife Edge, the Line of Draw, and the Alignment of the Paper in the Mount

The cork with the paper section is fixed to a slide treated with Mayer's albumin fixative, the paraffin is dissolved with xylene, and the cork is teased from the slide with a dissecting needle, leaving the paper section intact; the section is placed in a 95% alcohol bath to remove the xylene, and finally mounted in diaphane, or it may be mounted directly in Canada balsam. This method requires about three hours, using prepared corks.

Preparation of sections by inserting a strip of untreated paper into an impregnated cork and cutting with a hand microtome gives only fair results. In this instance, best results are obtained by leaving the paper section in the cork when mounted, since attempts to remove the cork section usually tear the paper section or pull it loose from its fixed position on the slide. This method requires about 20 minutes.

Cork Sectioning of Waxed Paper (77)

The paper is cut into a ribbon 4-5 mm. wide and about 5 cm. long. Two ribbons of nonmoistureproof cellophane are cut across the grain, the same size as the strip of paper. With a razor blade, a slit is made parallel to the long axis and at least halfway through a new No. 1 cork. The waxed paper is placed between the two cellophane strips and inserted longitudinally in the slit. The projecting ends of paper and cellophane are trimmed flush with the ends of the cork and the whole is mounted in the microtome in the same manner as described in the cork method.

The object is set for a 15 μm. section. As the knife passes over the cork, it does not cut a section of cork because of its resiliency, but does, however, cut a 15 μm. section of paper and cellophane. This is discarded and the knife edge is brushed free of adhering cork particles. To prevent separation of the paper section from the cork during the cutting a tiny drop of sodium silicate is placed at each end of the waxed paper section and allowed to dry. Now the knife will cut the cork as well as the paper.

The section is floated on a drop of water on a slide and the cork and cellophane are removed from the paper section with dissecting needles. After the paper section has dried in place on the slide, it is covered with a drop of 0.25% aqueous Crosein scarlet. Within a few minutes, most of the dye is removed from around the section and the remainder carefully blotted up with a piece of filter paper. The paper section is mounted with sodium silicate.

Paraffin Method (77)

The paper is treated in the same manner as for cork sectioning. The strip of paper is removed from the xylene solution of paraffin and immersed in melted paraffin for 30 minutes. The temperature of the paraffin bath is then lowered to just above the melting point. The paper strip is alternately removed from and dipped into the paraffin bath until a candle of the desired size is formed around the paper. It is cooled by dipping in ice water each time it is taken out of the paraffin bath, and finally trimmed for mounting in the microtome chuck.

The block is mounted in the microtome as described for the cork method. The angle of the slant of the knife is made as acute as possible, so that the largest amount of the knife edge available is presented to each fiber in the paper during the sectioning, thus reducing the tendency for the fibers to be pushed out of their positions.

Because the small angle of slant causes the section to roll, some means must be adopted to reduce this tendency. One method is to catch the sections on a camel's hair brush during the cutting. A thin film of collodion applied to the surface and allowed to dry, helps reduce rolling and also aids in keeping the fibers within the section in their proper position.

The section is floated on a drop of water on the slide to which the fixative has been applied, and warmed slightly to flatten the section and to remove the water. If collodion has been used in cutting the section, the collodion side is placed upward when the section is floated on the water. After the water is evaporated, the slide is placed in a bath of ether and alcohol to remove the collodion, immersed in xylene to remove the paraffin, and finally washed in alcohol to remove the xylene, after which the section is mounted.

Good sections 10 μm. thick are easily obtained by this method; the time required is about four hours. It is usually less trouble to cut across the machine direction of the paper than with the machine direction. Although alcoholic solutions of dyes seem to be more satisfactory than aqueous solutions in staining the paper section, the increased handling makes it seem inadvisable.

Sectioning of Wallboard (78)

Small blocks of various wallboards which have been softened by soaking in acetone are embedded in paraffin. Sections 30-50 μm. thick are cut perpendicular and parallel to the surface of the board. The paraffin is removed with xylene and the sections are mounted in Canada balsam.

Gum Arabic-Paraffin Method (79)

Kelsch has described a special technique for paper and inks which are not water soluble or dispersible. The embedding material consists of gum arabic, 50 g.; water, 83 ml.; glycerin, 25 ml.; and formalin, 10 drops. To prepare, first moisten the gum arabic with a few ml. of alcohol, then add glycerin, then the water and formalin.

The paper sample is cut into strips about 6-mm. wide and 5-cm. long. It is supported in a metal holder with a rod attached so that it may be inserted in a large test tube. Enough embedding solution is poured into the test tube to cover the sample. The test tube is then carefully evacuated and the evolution of air bubbles from the paper is watched. It must not proceed too rapidly. After 10-20 minutes the vacuum is broken and the sample placed in a small oven at 100°F. It is left in this oven until the embedding material is firm enough to just retain a fingernail impression. This may take 6-48 hours depending on ambient humidity.

The embedded sample is then placed in a paraffin frame and immersed. The block formed when the paraffin has hardened is trimmed to fit the jaw of any standard microtome. Sections are made one at a time and the embedded portion containing the paper section is delicately picked away from the paraffin and placed on a microscope slide. Several sections are so placed, and can then be immersed in oil and a cover slip applied.

Collodion-Paraffin Method (79)

Kelsch has also described a method for papers and inks sensitive to water. The embedding material is prepared from low viscosity nitrocellulose (in alcohol), 30 g.; ethylene glycol, 5 g.; ethyl or butyl acetate, 65 g.; and triphenyl phosphate, 15 g. The equipment and procedure are the same as given in the preceding section using gum arabic.

Collodion-Cork Method (77)

A paper strip 4 by 20 mm. is dehydrated in acetone. The acetone is heated slowly to the boiling point and boiled slowly until air bubbles cease to arise from the paper. The paper is transferred from the acetone to a 12% solution of cellulose nitrate in acetone. This transfer should be made rapidly, before the paper dries. The paper is kept in the collodion for one to two hours.

Two rectangular pieces of cork are prepared by cutting a paraffin-impregnated cork in half, trimming the sides to form two pieces each measuring about 15 by 8 by 2 mm., spreading a coating of cellulose nitrate on one face of each of the cork segments, and allowing them to dry.

The paper strip is removed from the collodion, one end is clipped in a wooden clothespin, and allowed to dry for 15 minutes. The strip is again immersed in

collodion solution (slowly, to avoid air bubbles), removed, and again dried for several minutes. The strip is then placed between the cellulose-treated faces of the cork segments and the whole fastened together with the clothespin to dry. If overnight drying is feasible, so much the better.

Finally, the paper and cork are mounted in the microtome, and 15 μm. sections are cut, using a camel's hair brush to reduce rolling. If the paper section splits during sectioning, this may be remedied by moistening the top surface of the mount slightly with acetone just before cutting.

The section is floated on a small drop of water and, with a little manipulation, can be sufficiently flattened. Most of the water is removed with a blotter or filter paper; a drop or two of acetone is placed on the section, and, as soon as the collodion has dissolved, the two cork sections are removed. The paper is mounted with diaphane before all the acetone has evaporated from the paper section, reducing the tendency for air bubbles to form within the section.

This method is also suitable for sectioning waxed paper. It is not necessary to leave the waxed paper strip in the solution for one to two hours for infiltration. Sodium silicate, water or glycerin should be used for mounting, as waxed paper cannot be mounted in diaphane.

Armitage (80, 81) recommends a similar method, but for tissue and unsized papers uses an addition of 2-5% camphor based on the weight of the undiluted cellulose nitrate as a plasticizer; for highly-calendered, coated or heavily-sized papers or stiff boards, the camphor may have to be eliminated. If it is desired, the cellulose nitrate slab can be embedded in paraffin.

Methacrylate Methods (82, 83)

More recently, embedding procedures which employ synthetic resins have been adapted from ultrathin microtomy. Marton (82) describes the following method for the preparation of paper cross sections. Paper strips, cut small enough to fit into a gelatin capsule (No. 00), were steeped in acetone for one hour and then transferred into a 50% solution of butyl methacrylate in acetone for one additional hour. Finally, the specimens were dipped into pure methacrylate and allowed to stand for one hour.

Following this sequence, the strips were fitted into the gelatin capsules, which were then filled with deinhibited methacrylate monomer purified by washing with alkali, neutralizing, and dehydrating. One percent of benzoyl peroxide was added as a catalyst. The capsules were capped and cured in an oven at 55-60°C. for 12 hours. After cooling, they were steeped in cold water for a few minutes; the softened gelatin was peeled off. The bottom and sides of the hardened methacrylate rods (in which the paper was embedded) were trimmed. Sections cut with the microtome were mounted in glycerin.

The following schedule with butyl methacrylate is used at The Institute of Paper Chemistry: Dehydrate in absolute alcohol for 15-30 minutes; place in a 50/50 mixture of absolute alcohol and butyl methacrylate for 30 minutes; impregnate with butyl methacrylate for 30 minutes; transfer to butyl methacrylate plus 1% benzoyl peroxide catalyst briefly and then orient the paper strip in a capsule and fill the capsule with a fresh solution of resin plus catalyst; put on top of capsule

and place in an oven overnight at 45-50°C. The inhibitor is removed from the resin by treating with dilute caustic soda, washing and neutralizing, and carefully dehydrating.

If the butyl methacrylate by itself proves to be too soft for best results, it may be necessary to add small amounts of methyl methacrylate to achieve the proper hardness. Methyl methacrylate, while it may be very good for ultrathin microtomy, is usually too hard and brittle for ordinary paper sectioning.

In 1942, Singleterry reported embedding the paper specimen in a transparent plastic (a urea-formaldehyde liquid resin containing zinc chloride was used, but methyl methacrylate was suggested), grinding and polishing one side, cutting off the polished portion with a jeweler's hacksaw, attaching (finished side down) to a microscope slide and grinding and polishing to the desired thickness of 10-20 μm. (84).

Epoxy Resin Method (72)

The epoxy resins have also been used for embedding paper for microtome sectioning (72). As discussed previously under embedding of fibers, Epon 812 has proved to be a very satisfactory embedding resin (70).

Polyethylene Method

Banks (85, 86) has recommended the use of a low-melting point polyethylene for embedding paper for sectioning. At no time during the preparation should the temperature be allowed to rise above 120°C. As soon as maximum transparency has been achieved the molds are allowed to cool and the solid blocks of polyethylene are trimmed around the paper strip to fit the object holder of the sliding microtome. Polyethylene exhibits birefringence, which may be a disadvantage.

Centola and Taraschi (87) described an embedding method utilizing a mixture of 25 parts polythene (low or medium mol. wt.) and 150 parts beeswax. The ingredients are melted together and then allowed to cool to 65-70°C., before pouring into a vessel containing the small paper specimen. Normal procedure for paraffin treatment is followed thereafter.

Gaiser (88) described a technique for embedding coated papers in a mixture of microcrystalline wax-polyethylene for cross sectioning.

Freezing Method

The freezing microtome, so commonly used in clinical laboratories, has scarcely been employed in botanical microtechnique because plant materials do not lend themselves readily to the customary freezing methods (41). But unembedded tissues that are too soft or fragile to stand up under the impact of the knife can, in some cases, be frozen and then sectioned. The device used on the sliding microtome for this purpose is known as a freezing attachment; it replaces the object holder of the microtome. Freezing is accomplished by the evaporation or expansion of a freezing agent – ether, carbon dioxide (gas or solid). According to Sass (42), the piece of tissue to be frozen is usually enveloped in a fluid or semifluid medium, such as gum arabic or gelatin, which on freezing affords additional support.

Jayme and Harders-Steinhäuser (89) have sectioned paper with a microtome of the carbonic acid-freezing type with a separate knife deep freezer. Use of the freezing microtome is greatly facilitated by a combination with the electrical cooling unit, "Frigomat" (manufactured by the Jung Company, Heidelberg). This accessory instrument, which corresponds to the cooling unit of a refrigerator, makes the freeze-cutting independent of carbon dioxide cooling (83).

In most cases, ice is a suitable embedding medium (83, 89); only very sensitive paper types, such as crepe, require embedding in paraffin prior to freezing. One difficulty with water is its swelling action. For this reason compact materials such as plastics, impregnating materials, and certain special papers are best suited for cutting on the freezing microtome. It is possible to discern very thin laminations on aluminum foil (83).

PARALLEL SECTIONING OF PAPER

There are times when it is desired to know the variation in composition throughout the thickness of a sheet of paper or paperboard. A technique for sectioning parallel to the plane of the sheet, which did not use an embedding matrix, was developed by Browning and Isenberg (90). A small piece of the sample was glued with Duco cement to a small block of a soft wood, held in the microtome object holder, which had been smoothed with the microtome knife. Layers of the sheet could be obtained which were 20-30 μm. thick. Although extremely thin sections could not be cut by this method, at the same time there was no possible interference with the chemical nature of the paper under study. Somewhat later a similar technique was reported (91).

A mixture of 40 parts polyethylene (low mol. wt.) and 60 parts microcrystalline wax may be used to embed newsprint after the sample has been glued with a water-soluble adhesive to a smoothed block of wood held in the microtome object holder (92). Serial sections 12 μm. thick may be cut if desired. The sections are handled in a manner similar to those in which ordinary paraffin embedding techniques are used. Other types of paper can be sectioned in this way.

A technique has been described for the preparation of longitudinal sections parallel to the axis of fibers, films, and other materials, which permits samples to be cut 1-2 μm. thick (93).

Oblique Sectioning

It has been proposed recently that paper or paperboard be mounted on a small block of wood smoothed in a microtome, tilted one half to a few degrees, and thus sectioned obliquely or slantwise to the plane of the sheet. The cut sections are then stained using different methods, depending on the constituents of the sample (94).

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PHOTOMICROGRAPHY

For many purposes a photomicrograph is more satisfactory than a line drawing or a verbal description. It does represent a selected field, however, and so it is a supplement and not a substitute for visual examination. Also, the camera, unlike the eye, has no power of accommodation, so a shallower field is recorded on the film than is seen under the same conditions by the eye.

FUNDAMENTALS OF PHOTOGRAPHY

The ready availability (1-5) of information on the fundamentals of photography precludes the need for detailed discussion here. The optical principles of the microscope are discussed earlier in this book.

FILMS AND PLATES

Successful photomicrography depends to a great extent upon the selection of film emulsions and developers that will attain optimum results under given conditions. Films with several different photographic characteristics should be at hand, and one should be familiar with many different developers suitable for special circumstances.

The choice of photographic film or plate has an important influence on the quality of the picture. For black-and-white photomicrography, color sensitivity, contrast, resolving power, and speed must be considered in the selection of a suitable negative material. Color sensitivity is inherent within a given material and is not subject to change by processing manipulation as are the others. Contrast and resolving power are usually of prime importance. Speed is of lesser importance since specimens are usually motionless (2).

Generally, the best procedure to follow in selecting a suitable material is to consider the quality of the specimen and how best to record its image. For ordinary work, fine grain contrasty process emulsions are preferable. When a specimen contains a variety of colors, a material sensitive to all visible colors must be selected. Panchromatic emulsions are indicated where there is a large amount of red or orange in the specimen, while use of yellow or green filters or presence of these colors in the object calls for orthochromatic emulsions.

Photographic materials should be selected with the highest contrast range available, without undue sacrifice of exposure latitude. Examples of this principle are Kodak "M" and Kodak Metallographic plates, made especially for photomicrography. Both can be used to produce negatives of low, medium, or relatively high contrast by proper selection of the development conditions. They also possess a reserve of resolving power over that directly used by the eye in a contact print or even with some enlargement. The "M" plate is a panchromatic plate, sensitive to all colors, and can be used in the most general case. The Metallographic plate is orthochromatic but otherwise rather similar to the "M" plate.

Most technicians prefer to use film despite the greater dimensional stability of plates. The best general-use film is Kodak Plus-X Pan Film, which has extremely fine grain, excellent exposure latitude, and is capable of a wide range of contrast by variation of development. If the high contrast of the "M" plate is needed, Contrast Process Panchromatic Film is suggested. Instead of Metallographic plate, Contrast Process Ortho Film is recommended. In roll film the most important characteristic is low graininess, since the images must often be enlarged many times (2).

Color Film

The choice of a color film for photomicrography is limited since the purpose of such a material is to record an image as it appears. Hence, deviation from standard practice often results in unpredictable contrast, speed, and color balance.

One factor which must always be considered when color films are used in photomicrography is illumination quality. Color films are designed to render normal color balance when exposed to illumination of specific energy distribution. If a light source burns at a color temperature which differs from that for which the film was designed, the photographs will be off-color unless appropriate filters are used in the illumination beam.

The amount and quality of filtering depends upon the difference in color temperature. Most light sources used in photomicrography burn at color temperatures below that for which artificial light color films are corrected. Color films most often used in photomicrography are those designed for use with artificial illumination since the difference in color temperature is less than if daylight type films are used (1-4).

GAMMA

In photomicrography, particularly where the detail or particles are discrete, a gamma higher than one is indicated. Gamma can be controlled by regulating the time of the development, by changing the temperature or nature of the developer, by changing the character of the emulsion, or by changing the spectral quality of the light.

Certain kinds of film lend themselves readily to increase in gamma by development, and great contrast can be attained with them. Gamma and contrast are not synonymous, however, and under certain conditions the contrast may be increased tremendously without changing the gamma. On the other hand, when gamma is increased, contrast increases also, for density of the negative is built up in the highlights faster than in the shadows. This sensitivity to increase in gamma is important in selecting sensitive material for photomicrography. An added tool for strengthening detail and improving visibility is the difference in the effectiveness of different developers in controlling gamma (3).

DEFECTS OF NEGATIVES

Occasionally, despite every precaution, the negative is not up to expectations. If it is generally too thin or too dense, the problem is usually improper exposure or development and may be corrected accordingly. Other common causes of defects in negatives are light or chemical fogging, improper agitation in the developer, too high temperature, overage solutions, inadequate washing, dust, and poor housekeeping in the darkroom. Many difficulties are avoided if directions are carefully followed and top-grade materials used.

FILTERS (3)

Filters are used primarily to modify and regulate the intensity of the light; to increase or decrease contrast in a specimen by selective filtration of the light; to aid in the performance of the optical components, and the eye; and to furnish polarized light.

There are three distinct types of filters: glass, gelatin, and liquid. Gelatin sheets are mounted between glass plates, including Polaroid plates. Liquid filters are made by filling flat-sided glass cells with plain water, sodium nitrite solution, or a colored liquid; they are valuable chiefly to absorb heat and radiation when an arc lamp source is used.

The filter is placed in the path of the light beam in such a position that the optical corrections are not of paramount importance. The filters are made of clear glass and light rays pass through them unaltered in direction with respect to each other and without diffusion. A white glass filter with a ground or matte surface diffuses and also partly absorbs the transmitted light; it is often placed close to the lamp as a secondary light source. Except for this diffusion plate, the best position for filters is midway between the lamp and the microscope.

A neutral filter absorbs all wavelengths of white light equally, or nearly so; only the intensity is reduced. A set of neutral filters with various densities should be on hand for regulation of light intensity. Some so-called neutral filters are so selective in transmission that combinations will result in light that is noticeably colored; it is usually made reddish. With certain types of illumination the neutral filter can be replaced by one or more diffusing plates at the lamp.

A color filter absorbs selectively for some wavelengths so that the transmitted light appears colored. The selection of chromatic filters can be based on the study of the spectrophotometric curves and on visual inspection of their effects on the specimen. (Eastman Kodak's book on Wratten filters and the catalogs of various glass companies furnish considerable data.)

If the specimen is colored, various filters should be tried, and the one giving the best optical effect should be selected. Filters of the same color as the object will give the greatest degree of transparency in the image. Filters complementary in color will give the greatest contrast. Filters that transmit light similar in color to the object increase the visibility of detail within the objects; however, in black-and-white print, the background will appear very weak

since it will lack contrast with the object. If none of the background appears, a filter of the same color as the object may give the best results.

A monochromatic filter transmits light of only one color, of a narrow band of the spectrum, and absorbs the remaining wavelengths. Satisfactory illumination by monochromatic light can be obtained by using the specified filters, and the mercury vapor lamp as a source.

In another form the chromatic filter absorbs the infrared and heat rays from the lamp; this special filter is known as a heat-absorbing filter.

Color filters may help to diminish chromatic aberration and to increase contrast in stained specimens. It is common practice with achromatic objectives to use a green filter for visual and photographic effects. Images of inferior quality will result if illumination of longer or shorter wavelengths is used; hence, the use of red, blue, or violet filters should be avoided with achromatic lenses if the best image is desired. Increased contrast is obtained if the filter transmits only the spectral zone most readily absorbed by the stain.

Since filters usually have low transmission values, the exposure time may be increased greatly. Fortunately, this increase in time is not so marked with filters within the visible range, but a filter used to give ultraviolet radiation to promote fluorescence might extend the exposure from several seconds to several hours. The filter factor is the number by which the normal exposure time is multiplied to determine the correct exposure time when the filter is used.

Any of the solvents can be used for cleaning solid glass filters, but generally warm water and soap is sufficient. Abrasives should be kept away from all types of filter, and when not in use these filters should be stored in the dark.

Light Balancing Filters

Color films intended for use with artificial light are generally balanced for use with tungsten lamps operated at a specific color temperature. In photomicrography, however, the most suitable lamp may not operate at the specified temperature and, without correction, may not be suitable for color photography. An electrical change in voltage or amperage may compensate but, if not, a special filter can be used to convert the spectral energy distribution of the illumination to very nearly the distribution of the tungsten lamp for which the film was balanced in manufacture. A number of these light-balancing filters are available since the actual sources may operate at any of a wide variety of color temperatures. They are available in gelatin form, mounted in "B" glass as squares or circles, and specifically for photomicrography they may be obtained mounted in 33-mm. very thin round glass circles which permit insertion in the filter receptacle which is found on most microscopes beneath the substage diaphragm (2).

Color Compensating Filters

It may be necessary to use these filters, either singly or in minimum combination, if the color reproduction shows slight deficiencies in the overall color balance. Such corrections are often required with unusual light sources, with optics which have selective absorption, or when heat-absorbing glass is used in the optical system (2).

Many types of cameras are available for photomicrography. These are commonly grouped into three classes: The existing camera, which has its own lens; the so-called "eyepiece" camera, with a fixed film plane; and the view-type, with variable bellows. Cameras designed for photomicrography are usually more satisfactory than those adapted for convenience. While the camera cannot improve on the image formed by the microscope, it is important that this image is not made poorer by the camera technique.

INTEGRAL LENS CAMERA

If only an occasional photomicrograph is needed, it may be simplest to add an existing camera to the microscope. This may be a box type, folding type, or 35-mm. type camera and usually uses roll film. The focus is set at infinity and the aperture is used fully opened. The camera may be positioned over the microscope by means of a laboratory ring stand and clamps, but specially designed adapters are available for certain cameras. The adapter contains a beam-splitting prism which divides the light into two beams, one continuing directly to the camera and the other diverting at 90° to a telescopic eyepiece for individual focusing by means of cross hairs. For the best image quality and field size from a camera with integral lens, the front surface of the lens should be placed at, or very near, the eyepoint of the microscope (2).

FIXED LENGTH CAMERA

One type of camera designed for photomicrography is the eyepiece camera, in which the film plane is in a fixed position, usually far enough from the microscope eyepoint so that only the central area of the field will be recorded. Most of these cameras are equipped with a beam-splitter eyepiece so that the image can be observed before and during photography. These cameras usually use 35-mm. roll film, but some use sheet film, and others will accommodate either kind. Despite the greater expense, sheet film has several advantages over 35-mm. roll film, such as larger negative size and individual development (2, 6).

ADJUSTABLE BELLOWS CAMERA

Vertical Camera

A bellows camera is normally employed when ability to vary the final projection distance, and hence the magnification, is required. The most useful type for covering a wide range of work is the 5- by 7-inch vertical camera. The camera contains a shutter capable of a range of exposure times, a light trap between the eyepiece and the shutter, and a ground glass screen for focus and composition. No camera lens is used since it might degrade the image by introducing reflections or aberrations. It is best to position the shutter so that its blades are located at, or very near, the eyepoint of the eyepiece. Although sheet films or plates in appropriate holders are usually used, some cameras will accommodate roll films by means of suitable adapter backs.

It is more convenient and time-saving to have the camera-microscope apparatus set up as a permanent unit but for occasional photomicrography a temporary arrangement will suffice (3). Leveling the equipment is important.

Horizontal Camera

In certain types of work, such as taking a number of photomicrographs at magnifications which permit the use of dry lenses throughout, it may be more convenient to arrange the light source, microscope, and camera in the horizontal position. Furthermore, this position makes it easy to achieve proper alignment since the whole assembly can then be mounted on an optical bench.

OPTICAL ALIGNMENT AND ILLUMINATION

ILLUMINATION

The importance of illumination in the production of ideal photomicrographs of transparent preparations is often overlooked. This is mainly responsible for the mediocre results so often achieved, rather than the quality of the optical system (1).

Illumination requirements essential to the best performance of a photomicrographic setup are:

1. The light rays should be symmetrically disposed about the optic axis of the microscope, and should be capable of entirely filling the area of the back lens of the objective, yet not be in excess of this.

2. The diameter of the illuminated area should cover the entire field of view to be photographed and means should be available to circumscribe it as near as possible to this area.

3. Uniform intensity is important throughout the entire illuminated area.

Centering the Filament Image to the Mirror

By any system of illumination, with the light source properly centered in the lamphouse, an image of the source is projected directly into the center of the microscope mirror. To do this, the mirror is turned so that its surface is normal to the optic axis of the lamp lens. Lack of centration reduces the light and makes it uneven in the object field, an undesirable condition in photomicrography (3).

Focusing the Brightfield Condenser

The condenser must be focused on the light source before being centered. The condenser is focused when its position is such that an image of the light source (either the ground glass at the lamp or the lamp diaphragm) can be seen in the field of view when the microscope is focused. An image of the light source must then lie in the object field.

To focus the medium and high-power condenser in the simplest and quickest way, the microscope is focused on a slide of well-dispersed discrete particles. The mirror is tipped slightly until the edge of the field diaphragm enters the view. At the same time this diaphragm edge can be focused by manipulating the condenser substage adjustment. If the lamp diaphragm is closed to a few millimeters in diameter, it can be used to focus the condenser. After focusing, any further motion of the condenser, either up or down, will make the image of the source increase in size.

Precision of focus is of less importance for uncorrected and low-power condensers than it is for those of high-power and good correction. Low-power condensers tend to show the structure of ground glass surfaces at the lamp more plainly than those of high power, since the aperture of such condensers is less and the contrast of the ground glass detail is enhanced. If this detail interferes with the background of the photograph, the condenser may be raised slightly or a second piece of finely ground glass added at the lamphouse.

Centering the Brightfield Condenser

Alignment of the condenser and the objective is very important. The camera can register differences in field intensity that the eye cannot see, and that frequently result in light spots or shadows across the film.

With a focused microscope and focused condenser, the first step in centering the condenser is to remove the eyepiece and insert a pinhole cap; next, inspect the rear focal plane of the objective where the image of the condenser diaphragm will be seen. The iris should be adjusted until the condenser is given about 9/10 cone of light. Then, by making the condenser circle concentric with the objective circle by manipulating the condenser centering screws, the condenser can be centered to the axis of the objective. The centering operation is aided by manipulating the iris diaphragm (3).

GLARE

In a microscope system, where light must be handled with exactness, extraneous light from the surroundings or light which has deviated from its planned course within the microscope is very likely to cause trouble.

Glare may affect the field of view or the image on the film in any or all of the following ways: 1. The image may be flooded with light and visibility reduced so a flat negative or a very weak field of view may result. 2. Small details or even small particles may be rendered entirely invisible. 3. Color effects may be made uncertain. 4. Unequal distribution of light over the film may give a spotty or uneven appearance to the negative.

Low-power objectives are less sensitive than high-power ones to conditions which may produce glare, partly because with the low power the image is so much stronger than the glare that it is not materially affected. With high-power objectives, control of glare plays an important part in securing a clean, brilliant negative.

Glare is conveniently classified according to source, and recognition of the source will generally suggest the remedy. The most important sources, in decreasing order, are an improperly prepared specimen, maladjustment of tube length, under- or overcorrected objective, improper adjustment or a poor or uncorrected condenser, and diffusing plate placed too close to the microscope.

Other possible sources of glare are the unavoidable reflection of an appreciable amount of the incident light at each lens surface, the cover glass, the surface of the microscope slide, too large a light source, lack of proper optical filters, the second-surface microscope mirror, improper adjustment of the iris diaphragm of the condenser, a condenser unsuited for the objective, extraneous light from the lamp or other source, light reflected from the inside of the camera bellows or from the microscope tube, halation caused by light reflected from the back surface of the plate or film, and a diffusing plate at the lamp (3).

GENERAL PHOTOMICROGRAPHIC PROCEDURE

LOW POWER

For low magnifications (5-50X), photography may be carried out using the objective alone; this is commonly known as photomacrography. These special objectives consist of photographic lenses of the usual fundamental design but computed for image distances of the order of photomicrographic bellow lengths, and are sold with various focal lengths as Micro Tessars, Mikrotars, Micro Summars, etc. The central definition of the lens is somewhat less than the corresponding microscope objective, as it is designed to have a relatively large field. The maximum aperture is about f/4.5 (about N.A. 0.1 for magnifications above 5 diameters), although it usually contains an iris diaphragm.

If extremely fine detail exists in the specimen, it is necessary to use the higher definition of the compound microscope above a magnification of about 30 diameters. When illumination by incident light is used, the light scattered by the specimen, unless the specimen is polished, is usually depended upon to fill the aperture of the objective.

MEDIUM POWER

At higher magnifications objective and eyepiece are used, the combination resulting in improved resolution and better correction of aberrations. For medium magnifications (100-500X), 16-mm., 8-mm., and 4-mm. objectives and 6X and 10X eyepieces are a useful combination. Apochromatic objectives and compensating eyepieces are preferable for color distinction, although achromatic lenses can also be employed with light filters to reduce chromatic aberration. Apochromats perform most efficiently with the light transmitted by appropriate green or blue filters. A flat image is necessary for good photomicrographs, and special flat field objectives and eyepieces are now available.

MAGNIFICATION

The magnification is determined exactly by photographing part of the scale of a stage micrometer. The apparatus should be calibrated for various camera extensions in conjunction with various lens combinations, and the results compiled as tables, or graphs, for ready reference. As far as possible, photomicrographs should be made at the standard magnifications of 10, 25, 50, 75, 100, 200, 500, and 1000X.

CLEANLINESS

The production of good photomicrographs requires the highest standard of cleanliness of slides and lenses. Small specks of dust in the mounting medium or on the lenses spoil the photograph regardless of how well other conditions may be satisfied. The location of dust particles can be determined by moving eyepiece, objective, and slide in turn and noting when the particles move. Time spent in cleaning equipment and care taken in preparing slides are well rewarded.

VIBRATION

Vibration must be avoided in order to produce sharp photographs. The ideal location for a photomicrographic outfit is in a place where there are no vibrations from the outside and it is directly part of a massive support. If this is impossible, it is advisable to mount the entire outfit on a suitable absorbent for the vibration such as a sprung table, or alternatively, a pad of felt or sponge rubber should be placed under the instruments.

The act of making the exposure may cause vibration, and, if a mechanical shutter is used, a cable release should be employed. After manipulating the microscope or drawing the dark slide of a film holder, it is best to wait a few seconds, when possible, before making the actual exposure.

PROCEDURE WITH VERTICAL CAMERA

The appropriate eyepiece having been selected, and the light trap for connecting the microscope to the camera having been installed, the front board of the camera is lowered, and other necessary adjustments can then be made. In order to return the microscope always to the same position relative to the camera, the microscope may be pressed against the stops provided on the camera base.

All illumination can be aligned by focusing the image of the source at the center of the mirror and making the spot of light central on the ground glass of the camera. The lamp filament must be centered with respect to the light collecting lens of the lamphouse. Then the image of the filament is centered on the mirror and the diffusing plate inserted into the filter holder of the lamphouse. The diffusing plate acts as a secondary light source. It should be located about

15 inches from the mirror, since this appears to be the point at which the lessening of glare, due to the diffusing plate, becomes negligible for further increase of lamp distance.

The field diaphragm of the lamphouse should then be closed to a small opening and, by turning the mirror, the spot of light is brought into the field of view. First, however, it is necessary to have the microscope focused on the stage micrometer, or other convenient object. The condenser must also be focused until the image of the spot of light is as sharp as possible. The field diaphragm of the lamphouse is then opened to coincide with the field of view. If the structure of the diffusing plate is visible in the field of view, the condenser should be raised slightly above good focus. The projection type of lamp is best for use with illumination by this method.

In the Köhler method, the image of the source is focused in the plane of the iris diaphragm of the substage condenser, as in the preceding method. The microscope condenser is then adjusted to focus the image of the lamp diaphragm in the plane of the object field. The back lens of the objective will be filled with light of even intensity, and an image of the lamp diaphragm can be formed in the field of view whenever this diaphragm is sufficiently closed. The image of the lamp filament must be made large enough to fill the microscope condenser with a solid field of light. This method should be used when it is necessary to eliminate the last vestige of visible glare or when fine structure is being studied at high magnification. In order of convenience, the lamps best suited for this method are the tungsten-ribbon filament, the 6V single coil filament, the Pointolite lamp, the H series of mercury-vapor discharge tubes, the photomicrographic GE lamp, and the carbon arc lamp.

A modification of the Köhler method is easily made by using a source of light from a projection lamp. With a diffusing plate in front of the source the lamp lens focuses the surface of the diffusing plate in the first focal plane of the condenser. In its best form, this method includes a small diaphragm over the diffusing plate, so made that its image just fills the front lens of the condenser with light. Any increase of the size of the diaphragm will not give additional effective light. Although the lamp life may be shortened somewhat by restricting the circulation of air in the lamphouse, the brightness in the object field is doubled (3).

PROCEDURE WITH HORIZONTAL CAMERA

In using a horizontal camera the absence of the mirror simplifies the centering of the optical components. The lamp can be focused on the center of the substage and the image of the lamp diaphragm will be formed on the ground glass screen of the camera. The introduction of an accessory lens, diaphragms, or filters should not cause disruption of this first step in centration. Since the use of an optical track makes lateral alignment quite easy, the only adjustments of importance are those of vertical displacement, or displacements along the illumination axis.

With the comparatively long horizontal bench it is possible to place a very strong light source several feet from the microscope and yet, by a properly selected system of intermediate lenses, illuminate a very large field. The light

is often carried as a parallel beam for most of the distance, the last lens acting as the secondary source. Focal points can be determined by inserting a black card or diffusing plate in the beam. The general effect on the pencil of light can be foretold from the shape of the lens.

A point source of light is desirable, such as a 6V 48W projection lamp, or a 100 c.p. Pointolite. Owing to the increased strength of the source it is often necessary to eliminate heat rays by an infrared glass filter or a cuvette of water placed before the lamp. Uniform illumination is essential and is achieved by correctly centering the optical system so that the whole objective is used, thus giving an even background. Be careful to protect the eye from the intense illumination at all times!

FOCUSING THE CAMERA IMAGE

First the field is examined in the ground glass and the image made as sharp as possible. The focus is completed with the aid of a focusing glass (4-12X). The glass need not be held exactly in the image plane, but it should be near to it. Better images are obtained with the focusing glass when the ground glass back of the camera is removed. With practice, the aerial image may be picked up easily and quickly in this way. The camera will record sharply much less field depth than the human eye; this may account for certain disappointments and the inability to obtain critical sharp negatives. A further method of focusing uses an adjustable sidetube containing an eyepiece with a graticule. The latter is adjusted to coincide in focus with the image on the ground glass screen located in the plane of the photographic film. If the bellows length remains fixed, focusing can be carried out via the lateral eyepiece in place of the ground glass screen.

EXPOSURE

Several factors control exposure time: intensity of the light source; spectral quality of the light source; modifying light filters; focal length, N.A., and type of condenser; focal length, N.A., and type of objective; focal length and type of the eyepiece; distance from the eyepoint to the image field; the sensitivity and nature of the film emulsion; the nature of the specimen; total absorption of light by all the lenses in the system; adjustment of focus and aperture diaphragms of lenses.

The most direct way of learning the proper exposure time is to make a test film. Four or five exposures of graduated timing, or even more, can be made on one 4 x 5 or 5 x 7-inch film, the steps being in geometrical ratio. A suitable portion of the specimen must be included in each step, otherwise it will be impossible to form an adequate opinion of the correct timing. The test film will also serve as a check on the distribution of the specimen in the field, the coverage of the film, magnification, resolution, field depth, and contrast, as well as determining development time and other values. Generally, the final exposure time should be somewhat shorter than that deemed to be correct time on the test strip by inspection.

No one plate or film is capable of dealing with all the gradations of contrast encountered; neither can one developer or one time of development satisfactorily handle all negatives. Hence, the interpretation of the test film is of great aid in determining exposure time, a proper developing time, and the appropriate developer.

Although there are now cameras which automatically determine the time for exposure, exposure meters have never been entirely successful in photomicrography. In the first place, the light intensity of the image field is so weak that often an ordinary meter will not register. Frequently, only background lighting strikes the photoelectric element.

COMMON FAULTS IN PHOTOMICROGRAPHY

At this point it appears desirable to list the main difficulties which lead to poor results in photomicrography.

1. Poor and inaccurate focusing.
2. Vibration.
3. Instability of the fine focusing mechanisms during exposure.
4. Focusing with a filter of one color, or no color at all, followed by the introduction of another filter of a different color without refocusing.
5. Improper exposure or development.
6. Improper color filter.
7. Improper illumination.
8. Improper magnification.
9. Unevenness of background traceable to film emulsion.

PHOTOMICROGRAPHY OF FIBROUS MATERIALS

FIBERS

Unless an individual fiber, or an interesting part of a fiber, is being photographed, it is well to work at relatively low magnification so that a representative field is shown. Important information may be overlooked if too small a part of one fiber is all that appears in view. A 16-mm. or 8-mm. objective may be entirely satisfactory for most purposes. In general fiber work, it is rarely necessary to use an objective above 4 mm., so that special substage condensers of high numerical aperture are unnecessary.

Although many plant or textile fibers may be used unstained, it is usually better to stain them and use optical filters to bring out detail. This is helpful with pulps or macerates. Stains such as "C" stain, Congo red, and Malachite green are good. Chlorazol Black E has been recommended and used for photomicrography of pulp fibers. The modifications which fibers undergo during their treatment for papermaking are demonstrated in a convincing way by photomicrographs. In addition to brightfield illumination, it may be advantageous to consider special techniques, such as darkfield or phase-contrast, to show fibrils formed during

the refining process. Each case must be considered individually to emphasize the information one wishes to present to the viewer. Photomicrographs of pulp fibers, wood cells, and portions of them are shown in Chapter VI.

WOOD SECTIONS

Usually, best practice is to stain the wood sections and use suitable optical filters but if the specimen is deeply colored such procedure may not be necessary. The field of view is normally entirely filled with the section to be photographed but, depending upon small area involved, the amount of cell lumen showing can vary considerably. Magnifications commonly used are 50 and 100X, although others may be more suitable in certain instances.

Some workers photograph by reflected light, using a smooth surface on the wood block. It has been suggested that the cell lumens of cross sections be filled by rubbing with finely ground levigated alumina before photographing.

PAPER

Formation

By making direct photographic contact prints the formation of a sheet of paper can be compared with others. The paper is exposed in direct contact with sensitized photographic paper, then developed. Various characteristics of the sheet, such as degree of hydration, formation, opacity, wire marks, will be brought out. Watermarks are also recorded by this technique.

If desired, some of these sheet characteristics may be photographed at magnifications greater than one, using transmitted light. The sheet may be mounted in oil. A 30-mm. objective is useful in this case.

Surface Structure

Photographs of paper surfaces are usually made at magnifications less than 50 diameters, mostly at 5, 10, or 20X, and perhaps are best termed photomacrographs. Various Micro-Tessar objectives, such as 48, 32, and 24 mm., may be used for good paper surface photographs. A Tessar lens with a focal length of 50 mm. produces an image on the film about five times the diameter of the sample area.

The angle at which the light rays strike the specimen is a very important factor in both qualitative and quantitative appraisal of paper surfaces. The maximum angle between the paper surface and the incident light is, of course, 90°, and the minimum angle is slightly greater than 0.0°. Between these extremes lie an infinite number of choices. As the angle of lighting approaches 90°, even quite rough surfaces will appear quite smooth. As the minimum is approached, even the smoothest papers will appear quite rough. The sensitivity to surface irregularities increases as the angle is reduced. Grazing light at an angle of 5 to 10° from the surface, or 85 to 80° from the normal, is usually best for most purposes. To have any value for comparison between two paper surfaces, the lighting angle must be the same for each. The amount of shadow area, under

similar conditions, is a fair qualitative indication of surface roughness. The variable angle surface illuminator described in Chapter II may be of interest in this connection.

Paper Sections

In addition to the fibrous components, the photomicrography of paper or board cross sections may be influenced considerably by the nonfibrous constituents in the sample. The presence of a certain material may necessitate the use of reflected light instead of transmitted light, or a combination of both types, in order to bring out the details. Special staining techniques may be required also. Magnifications of 50, 100, and 200 diameters are used most frequently, but occasionally higher magnifications are desirable.

SPECIAL PHOTOMICROGRAPHIC PROCESSES

DARKFIELD

The use of darkfield illumination to bring out details, such as fibrillation in papermaking fibers, has been discussed. This type of illumination may also be helpful to highlight the nonfibrous constituents in the sheet or to locate small particles. The methods for obtaining darkfield illumination are described in Chapter II; the most common method for low and medium magnifications is an appropriately sized wheel stop in front of the substage condenser.

METAL SHADOWING (6, 7)

Two decades ago, metal shadowing in vacuum was shown to be a very useful technique in the preparation of specimens for electron microscopy (8). The use of gold shadowing was also recommended for the study of surface structure under the optical microscope. In shadowed specimens, details of the order 0.25 μm. may be seen, a degree of resolution which is not readily obtainable with much biological material owing to lack of contrast. Shadowing becomes a relatively simple method of using the optical microscope to the limit of its resolution.

In 1950, the author and co-workers (9), using aluminum, shadowcast Douglas-fir kraft pulp fibers (beaten for various time intervals) to bring out details such as fibrillation and pitting. The metal shadowed fibers were photographed with transmitted light. Using the identical field of view, a series of photomicrographs was prepared to compare the effect of shadowing with the unshadowed fibers under brightfield, darkfield, and phase-contrast (9).

Emerton and co-workers (10-12), a decade ago, emphasized the value of this technique for the light microscopical examination of dry fibers and of fiber and paper surfaces. Other workers have used aluminum metal casting and replication to study fiber surfaces by light microscopy (21, 22).

POLARIZED LIGHT

Examination with polarized light was discussed in Chapter III. The use of polarization may emphasize certain structures; in fact, without its use some details might not be evident or identifiable. In photomicrography the use of black and white film is often satisfactory, but in certain cases it may be worthwhile to use color film for added emphasis. At times reflected polarized light may be helpful (6, 7, 13).

ULTRAVIOLET MICROSCOPY

In addition to increasing the resolving power, a contrast greater than that which can be obtained with normal visible light is achieved with ultraviolet radiation, owing to differences in the differential absorption and reflection of the two types of illumination. This technique has been used with excellent results in various biological researches, but has not been applied to any great extent in fiber research.

The UV region of the spectrum begins at about 4000 A., corresponding to the lowest wavelengths detected by the normal human eye. Since a number of optical glasses are transparent down to 3000 A., it is possible to use achromatic objectives of normal construction for the range 3000-4000 A. A high-pressure mercury lamp (3650 A. line) is a convenient radiation source. The difference in focus of UV and visible light may necessitate the making of several exposures in order to determine the correct adjustment. A series of exposures is made on one film, using a suitable mask. The position of the fine focusing control is noted each time and the final photomicrograph taken at the optimum focus level.

With radiations less than 3000 A., lenses, slides, etc., must be made of special materials such as fused or crystalline quartz, fluorite, or lithium fluoride. The objectives are usually corrected for cadmium 2750 A. and 2570 A. lines, or the mercury 2536 A., and at high magnifications image definition rapidly deteriorates if the wavelength for which the system has been corrected is departed from by more than some 20 A. This defect has been mitigated by the development of lithium fluoride-fused quartz achromatic pairs. Below 2000 A. UV radiation is absorbed by the atmosphere and special vacuum apparatus is required. For the region 2500-3500 A., which is that most commonly used, this difficulty does not arise.

In addition to special lenses, UV microscopy requires reflecting surfaces based on evaporated aluminum films deposited on the front surface of the mirror. These strongly reflect UV radiations of all wavelengths in common use. Specimens are mounted in water, castor oil or glycerol-jelly, while immersion fluids are glycerol-water mixtures, or glycerol-sucrose solutions, the latter being less sensitive to atmospheric humidity. Rough focusing is done with a fluorescent screen or fluorescent eyepiece, the latter being fitted with an absorptive filter to screen the eye from direct UV rays. The final record is made photographically (6, 7).

FLUORESCENCE MICROSCOPY

It is convenient to discuss fluorescence microscopy at this point but it should be noted that the process differs from UV microscopy in that the image arises from visible light and is not a record of the UV absorption of the specimen. Hence, one should not expect increased resolution.

A typical system for fluorescence microscopy employs a 250W high-pressure mercury vapor lamp as a source, in combination with a lamp condenser, water trough and deep blue filter or a 20% copper sulfate solution. Thus, red and infrared rays are cut out and illumination is confined to the far blue and near UV region of the spectrum. No special quartz components are needed to transmit these wavelengths. Obviously, the light path should be entirely free from fluorescent materials, such as fluorite objectives. Specially selected slides, cover glasses, and lenses must be used, and mounting and immersion media should be based on glycerol and water (Canada balsam and cedarwood oil are fluorescent).

The specimen is illuminated in the usual way with the substage condenser at maximum aperture. A yellow filter which permits the passage of the fluorescent light but absorbs all visible and UV rays from the background is placed in the eyepiece to protect the eyes. Lamp and microscope screens give further protection.

The specimen may exhibit auto-fluorescence or the effect may be a secondary one arising from fluorescent dyes. Primary fluorescence is of greater fundamental importance as indicating a definite chemical property of the specimen. The use of fluorescent dyes has been described by numerous biologists. These dyes increase contrast as do stains in visible microscopy.

Cellulose shows little fluorescence; lignified fibers exhibit an intense green-blue fluorescence. Undamaged wool does not show fluorescence but the damaged fiber treated with fluorescent dyes shows fluorescence of secondary nature (6, 7, 13, 14).

INFRARED MICROSCOPY

There are several factors to which special attention must be given for photomicrography in the infrared region. Of course, the source of radiation is selected so as to have ample energy in the region to which the films respond, filters being used to confine the exposure to the desired spectral region. Infrared absorbing stains are used or not, according to the contrast and detail desired. The infrared image is invisible so focusing represents an important problem (7, 15).

Although apochromatic objectives are greatly preferred, most infrared photomicrography is done at low powers, where only achromatic objectives are available. The chromatic aberration of achromats may require considerable readjustment of focus. Also, appreciable degradation of definition will occur unless a narrow spectral band is employed.

The sources of illumination used in photomicrography by infrared are the common arcs, tungsten filament or ribbon lamps, and the Pointolite. Exposure

time is determined by making a step series. The film holder must be opaque to infrared.

Focusing is performed by one of these methods: 1. visually in red light; 2. photographic calibration of the fine adjustment and application of a correction by focusing visually in green light and then making a series of exposures in infrared with changes in fine adjustment; 3. correction of fine adjustment based on curve of the residual chromatic aberration of several achromatic and apochromatic objectives at certain visible wavelength exposures.

Infrared photomicrographs should be compared with results obtained with the normal techniques using green light to demonstrate the advantages of the infrared. In some instances it would be desirable to compare the infrared results with those obtained by visible red light.

Infrared photomicrography has been applied with success to the study of cellular structure of plants. The cell walls, especially when colored, are often more transparent to infrared than to ordinary light, so that details in the cells not normally visible may appear very distinctly. Using neocyanin as a stain and infrared film having a maximum sensitivity at 8200 A., Fowler and Harlow (16) obtained good detail from thin wood sections. The compound middle lamella was resolved into the very dark intercellular substance and the adjacent primary walls.

Infrared may be important in the study of fibers and structure, where black and brown dyes have been used. Most dark dyes are transparent in the near infrared. In dyed wool, the scales and other structural details can be revealed. The effects of chemical finishing treatment of fibers can be studied.

PHASE CONTRAST MICROSCOPY

Phase-contrast functions by converting phase changes in the object into visible intensity changes in the image. Lens arrangements retard a portion of the light in the optical path (6, 7, 13, 17).

Bright contrast has been useful in studying the structure of cotton fiber, while the structure of the cell wall and contents of the lumen of ramie show more clearly than with the ordinary microscope. The skin and core of rayon yarn fibers and their cross sections are differentiated quite clearly when mounted in paraffin oil. Surface saponification of cellulose acetate is readily visible because of the difference in refractive index from the normal cellulose acetate.

With papermaking fibers, staining reactions are more valuable for identification purposes. Nevertheless, phase-contrast is useful for showing the condition of the fibers, for estimating freedom from lignin, and in the examination of baryta and other surface finishing agents. Examination of paper sections may be of value in studying the composition of ink and for measuring ink penetration below the sheet surface.

Phase contrast will detect the boundaries of scales in sections of animal fibers possessing thick cuticles, which are normally invisible. Thin sections

are most suitable. Other studies on wool have been concerned with the detection of scale remains on damaged fibers, resin deposition, bacterial and enzymatic degradation.

INTERFERENCE MICROSCOPY

The required selective phase modifying action of the phase contrast optical system is more complete for fine structures and edges than for coarser and more gradual ones, so that the former are emphasized at the expense of the latter. In the interferometer, the interference effects which result from the combination of the beams on the observer's side of the object are not restricted to the finer, highly diffracting features, but reveal the coarser and more gradual features also. Another advantage is the facility which is afforded for measuring changes in optical path length introduced by the object. This may be related to the topography of reflecting surfaces or the amount of solid material in living cells (6, 7, 13, 18).

Interferometric technique is used to study the surface of textile fibers and slight differences in refractive indices. It may prove to be of value for examining the surfaces of the paper sheet.

CINÉ PHOTOMICROGRAPHY

The use of the motion picture camera with the microscope offers several advantages over still photography for certain types of work. The possibility of obtaining a continuous record of every phase of development of the subject with a minimum expenditure of time and the better opportunity for study which the researcher is afforded because of his ability to control the apparent rate of action of the subject by regulation of camera and projection speeds are the most important of these. With the aid of a beam-splitter the operator is able to focus the microscope and to select the best field of view without interrupting the photographic process.

There are certain essential points to be understood. The motor drive, the ciné camera, and the microscope are arranged on a common mounting board, with the illuminant in the best position beside the board. The microscope is fastened on a base plate resting on sponge rubber. It is absolutely necessary that the platform of the camera is level, that the optical axis of the microscope is perpendicular and that the horizontal optical axis is perpendicular to the film.

Exposure time depends upon the number of frames per second (depending upon the rapidity of motion), the different openings of the shutter sector, and the different values of the neutral screens. The product of the light and the time must always be the same for a given emulsion so there are only certain combinations of shutter and screen which can be used. For each intensity the shutter sector and screen are arranged separately for the different numbers of frames per second and selection of filters. The ideal magnification is that when all detail resolved is visible on the screen.

COLOR PHOTOMICROGRAPHY

The use of color film may have definite advantages to emphasize detail and contrast, as in the reproduction of colors of pigmented fibers and stained specimens, in the study of dyeing problems, and in polarization microscopy. In other instances, it is used only to make the photograph more pleasing to the viewer. Of course, the additional expense must be justified. Lighting and other equipment requirements may prevent the use of color. Due to their extensive color correction, apochromats are highly recommended for color photomicrography.

STEREOSCOPIC PHOTOMICROGRAPHY (19)

Stereograms are obtained by taking two photomicrographs from positions on either side of the normal on the center of the object and combining the prints or the transparencies. The centers of the two photographs are 2-1/2 inches (64 mm.) apart on the ground glass of the camera and equidistant from the center. The exposure time is the same; the condenser and illuminant are adjusted (1).

Readjustment of light can be overcome by one of the following methods:

1. With a sliding or centering nosepiece.
2. Use of a small eccentric circular aperture to cover first one half and then the other of the back lens.
3. Use of a two-objective binocular microscope.
4. Single-objective binocular microscope using cap diaphragms and substage stop.
5. Ordinary microscope photographing upper and lower focal plane — superposing the photographs.
6. Illumination first on one side and then on the other, with transmitted light.
7. Swinging microscope while holding object stationary; or swinging the illuminant, condenser, and stage at the center of the optical plane, and keeping the microscope objective stationary.
8. By tilting the slide on the microscope stage, using a special holder.

The stereoscopic picture will be sharper, the greater the depth of focus. Also, the greater the N.A., the better the picture. This leads to a compromise as these aims are incompatible.

The negatives are given full exposure, but relatively soft or medium development. Both negatives are developed at the same time in the same tray and without hand work. In trimming the prints, leave on the right-hand print 1/4 inch more than on the left side, and the same on the left on the left-hand print. Mount the two prints 1/8 inch apart.

Fiber Substances

Herzog (20) has suggested the following formula for displacement of the optical axis: $O = w \cdot f^2 / s \cdot b$, where O = the displacement, f = focal length of the objective, b = the width of the picture, s = the focal length of the stereoscope lens, and w = the pupil distance of the observer.

For small enlargements, swinging the camera right and left 3° on each side gives the best results.

Anaglyphs

A pair of stereograms is printed in two contrasty colors, superimposed and viewed with an anaglyphoscope, with red for the right eye and blue or green for the left. The simplest viewer is a cardboard mount with two openings covered with red and green cellophane or gelatin.

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